Pelorus





# Final Report

## Laboratory Microcosm Testing:

## Aerobic Biodegradation of PAH's & Vinyl Chloride

Prepared for:

**The Forrester Group**
444 Chesterfield Center
Suite 250
Chesterfield, MO 63017

Prepared by:

**Pelorus EnBiotech Corporation**
3528 Evergreen Parkway
Almquist Building
Evergreen, Colorado 80439

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# INTRODUCTION

## Background

UPRR has been conducting site activities under the CERCLA protocol at the Ogden Railroad located in Ogden, Utah. Two operable units that will be addressed in the Feasibility Study are the Northern Area OU-1 and the Ogden Rail Yard Groundwater (OU-4). Two different classes of contamination have impacted these two areas.

- **Northern Area (OU-1).** The focus of this operable unit is a DNAPL zone that is primarily composed of PAH's DNAPL impacted sediments exist in the 21st Street Pond, a local groundwtaer "sink".

- **Ogden Rail Yard Groundwater (OU-4).** Two chlorinated solvent plumes exist in the alluvial aquifer beneath the site. In each of these plumes, vinyl chloride is the predominant constituent of concern.

Contaminant fate and transport assessments have indicated that natural attenuation mechanisms are operting in these two operable units. This treatability testing ws performed to support analysis of remedial alternatives that incorporates natural attenuation.

## Objective

In support of the on going site remedial investigation, The Forrester Group subcontracted Pelorus Environmental & Biotechnology Corporation to perform a series laboratory bench scale tests. These tests were performed to determine the effect of oxygen supplementation on the natural occurring microbial processes that are currently contributing to the biological component of natural attenuation. For the purposes of these tests the primary constituents of concern (COC) are the low molecular weight PAH's, naphthalene, methylnaphthalene and phenanthrene and the chlorinated volatile organic compound, vinyl chloride. The microcosm tests that are the subject of this report were performed to evaluate aerobic biodegradation of COC for two locations:

- Soil and/or sediments within the vinyl chloride plume that are or that could potentially be impacted by future migration.

- Aquifer sediemtns being impacted by a DNAPL pool north of the facility, the dominant PAH constituents being naphthalene, methylnaphthalene and phenanthrene.

The microcosm testing performed by Pelorus Environmental & Biotechnology Corporation focused on aerobic biodegradation because the COC's (i.e., vinyl chloride, naphthalene, methynaphthalene and phenanthrene) are known to be biodegradable aerobically, and there is site evidence suggests that these contaminants are naturally attenuating to low levels in the aerobic fringe of the plumes. Microcosm tests were designed to support two types of remedial action alternatives for the above conditions:

- Active bioremediation of the plumes through delivery of rate-limiting reagents to the subsurface (i.e., oxygen as an electron acceptor).

- Natural attenuation of the plumes, including intrinsic bioremediation of the plumes as they discharge into aerobic groundwater zones in the immediate vicinity of the Ogden or Weber Rivers.

Because of the different test methods applied during the laboratory-testing program, this report has been prepared in five sections. Following is a brief summary of the report layout and description of the contents within each section.

**Section I:** Describes the preparation and characterization of site materials for the bench scale testing.

**Section 2:** Summarizes the analytical methodology used by the Pelorus project support laboratory to quantify the contaminants of concern during bench scale testing.

**Section 3:** Is a presentation of the procedures, results and conclusions of the preliminary screening tests used to guide the subsequent microcosm testing.

**Section 4:** Presents the procedures, results, and conclusions of respirometer microcosms for evaluation of enhanced PAH bioremediation.

**Section 5:** Presents the procedures, results, and conclusions of serum-bottle microcosm tests for evaluation of aerobic vinyl chloride biodegradation.

# SECTION 1: CHARACTERIZATION OF SITE MATERIALS

Sample collection for bench scale testing was performed by the Forrester Group. Sediment cores and groundwater samples were collected on June 11, 2002 from the designated locations within the DNAPL area and the Vinyl chloride areas of the plume. Sediment samples were collected from each location in two-inch plastic core barrel liners over a depth of approximated 2 to 3 feet. The ends of the core barrels were sealed with parafilm, capped, and taped closed with duct tape then shipped overnight to Pelorus Environmental & Biotechnology Corporation on ice. Approximately 2.0 liters of impacted groundwater was collected in unpreserved amber bottles with zero headspace. Bottles were secured in plastic bubble wrap placed on ice coolers and shipped on ice. All samples were received in good condition by Pelorus Environmental & Biotechnology Corporation on June 13, 2002. Table 1 below defines the sample designations as received from the field sampling team.

**Table 1.** Sample locations and designations of materials collected for bench testing.

| LOCATION | SAMPLE TYPE | SAMPLE DESIGNATION |
|---|---|---|
| DNAPL Area | Sediment | 33-B85<br>33-B86<br>33-B87 |
| | Groundwater | 33-MW6FP |
| Vinyl chloride Area | Sediment | 34-B75<br>34-B76<br>34-B77<br>34-B78 |
| | Groundwater | 34-OB12 |

Samples were stored and refrigerated upon receipt. Sediment cores for each location were split open and the contents screened through a 40-mesh standard sieve screen to remove large cobbles. An area composite sample was prepared with for each location with the final composite being placed in plastic lined 1.0 gallon paint cans that were sealed labeled and stored refrigerated at 8 to 10° C until needed. The oversize sediment

core reject material was rinsed with sterile water to remove loosely bound fine materials and associated contaminant and stored in amber bottles under refrigeration.

Sample characterization was limited to an analysis for target contaminant levels, and specific microbial population densities to determine the characteristics of the indigenous populations. Table 1-2 summarizes the results of baseline analysis of composite sediments for target constituents. PAH analysis was performed according to EPA method 8270 guidelines using a base/neutral micro-extraction protocol (see Section 2). Sediment vinyl chloride levels were estimated by headspace analysis of an aqueous sediment extract.

**Table 2.** Baseline contaminant levels in composite materials prepared for bench scale biodegradation testing.

| ANALYTE | DNAPL Composite (mg/Kg) | Vinyl chloride Composite (mg/Kg) |
|---|---|---|
| Total PAH | 35.00 | ND |
| Naphthalene | 7.28 | ND |
| 2-Methyl Naphthalene | 9.64 | ND |
| Phenanthrene | 5.8 | ND |
| Vinyl chloride | ND | 0.00178 |

Analysis of groundwater samples was not performed. This decision was predicated on the fact that groundwater samples from the site would be aerated and sterilized prior to use in bench scale tests essentially stripping volatile and semi volatile constituents. Therefore, baseline levels would be determined from the analysis of the sterile control initial time points of the respective bench tests.

Microbial plate counts were performed to determine the indigenous population densities of PAH (i.e., naphthalene, 2-methyl naphthalene and phenanthrene) utilizing and vinyl chloride utilizing microorganisms. Sediment samples (~10 grams) were suspended in 100 milliliters of sterile phosphate buffer (pH 6.5) and agitated for 15 minutes. The liquid phase extract was then subjected to a 10-fold serial dilution series out to the $10^{-8}$ dilution and

then plated onto mineral salts agar medium. For enumerating vinyl chloride degraders a low buffering mineral salt medium supplemented with a pH indicator dye was used. As vinyl chloride degrading colonies grow, they release the chlorine atom into the surrounding medium causing a localized pH drop (i.e., HCl production) that results in a color change of the medium around the growing colonies. Volatile substrates (i.e., naphthalene, 2-methylnaphthalene and vinyl chloride) were provided in the gas phase using gas tight chambers with an oxygen atmosphere. Phenanthrene degraders were enumerated by spraying an ethereal solution of PAH substrate over the surface of the inoculated plates generating a thin film of phenanthrene on the agar surface. Table 1-3 summarizes the results of these analyses.

**Table 3.** Summary of specific contaminant degrading microbial population densities.

| Substrate | DNAPL Composite Cfu/gram | Vinyl chloride Composite Cfu/gram |
|---|---|---|
| Naphthalene (NP) | $5 \times 10^5$ | ND |
| 2-Methylnaphthalene (2MNP) | $8 \times 10^5$ | ND |
| Phenanthrene (PHN) | $3 \times 10^4$ | ND |
| Vinyl chloride (VC) | ND | $2 \times 10^3$ |

The PAH degrading microorganisms produced good-sized colonies (2-3 mm diameter) on the agar medium with the specified PAH as the sole source of carbon and energy. Phenanthrene utilizers produced clearing zones around the colonies where the substrate had been solubilized and taken up by the colony for growth. Some of the colonies produced a tan to dark brown pigment resulting from degradation by products (i.e., catechol). Vinyl chloride enumeration was not straight forward as the colonies produced were very small (<< 1.0 mm diameter) colonies. However, some of these microcolonies produced a color change in the agar media immediately around the colony. This color change was visible before the colonies themselves could actually be visualized. Therefore, vinyl chloride enumerations are based solely on those colonies that actually caused a color change in the pH dye indicator.

# SECTION 2: BENCH SCALE ANALYTICAL METHODS

This section summarizes the sample preparation methods and analytical procedures used to determine the extent of target constituent biodegradation during bench scale testing. The analytical methods used by Pelorus have been developed and validated by the Pelorus Project Support Analytical Laboratory and are implemented under a Good Laboratory Practices program. Standard Operating Procedures are published for all test methods and are available upon request.

## Polycyclic Aromatic Hydrocarbon (PAH) Analyses

PAH constituents present in DNAPL area sediment samples where extracted using a micro-extraction procedure. The liquid content of the test systems were decanted into a separate zero headspace container and 5 to 10 grams of sediment where removed. The sediment was placed in a 40 ml extraction vial and mixed with an equivalent mass of anhydrous sodium sulfate. Fifteen milliliters of 10% acetone in methylene chloride was added, and the vial then sealed with a Teflon lined cap. The samples were then agitated for 1.0 hour on a rotary shaker to completely mix/disperse the sediment with solvent. The solvent was removed and placed in a solvent evaporation tube and the sediment was extracted with a second volume of solvent. The pooled solvent extract was reduced to approximately 1.0 milliliter of volume under a gentle vacuum sparge. The extract was added to a GC vial and the final volume adjusted to 1.5 ml.

Samples were analyzed on an HP5890 gas chromatograph with an FID detector. Analytes were separated on an Agilent DB-35MS capillary column (50 meter X 0.25mmID X 0.25 um film). A complex mixture PAH derived from coal tar (National Institute of Standards and Technology) was used as the Standard Reference Material (i.e., SRM 1597) to identify and quantify PAH constituents. A temperature program from 120°C for 8 minutes to 325°C at a temperature ramp of 5 degrees per minute was used to separate and elute constituents. Confirmation analyses were performed under identical gas chromatographic conditions using an HP5971 MSD (mass selective detector) to affirm peak identifications and retention time windows. A five point calibration curve was prepared using the PAH standard NBS

1597. A continuing calibration standard and a matrix spike was run at every time point with each batch of samples.

Liquid phase samples were analyzed by HPLC on a Varian Model 9360 system. Polycyclic aromatic hydrocarbons were eluted from a Supleco $C_{18}$ reverse phase column using an acetonitrile:water solvent system. Aliquots of aqueous phase samples were centrifuged to remove sediment fines and 1.0 to 2.0 milliliters of sample injected onto the column. PAH constituents of concern were separated and eluted from the column using a linear solvent gradient from 20% to 95% acetonitrile in water run over a 30 minute program. Constituents were detected by UV absorbance at a fixed wavelength of 254 nm.

## Vinyl Chloride by Headspace Analysis

Vinyl chloride is a gaseous substrate with a dimensionless ($mol*L_a^{-1}/mol*L_w^{-1}$) Henry's constant of 49.5 at $25^0$ C. Based on the contaminant fate and transport analysis performed by the Forrester Group the soil partitioning coefficient was calculated to be 0.004 L/kg for this site and therefore it is assumed that vinyl chloride is not appreciably adsorbed onto site sediments. Consequently, all vinyl chloride analyses were performed by headspace analysis.

Samples of headspace were removed using Hamilton Gastight syringes ranging in capacity from 100 uL to 2.5 ml in volume. The sample volume was a function of the headspace concentration of vinyl chloride. Typically, a 1.0 ml volume of headspace was flushed through the syringe by pumping the syringe three times and then injecting the 1.0 ml headspace sample directly on column. If the concentration was above the upper calibration limit then a smaller volume would be injected. In some instances, it was necessary to dilute the headspace by 100-fold in order to quantify the vinyl chloride levels. In these instances a 250 ul headspace sample was collected in the 2.5 ml syringe and diluted (10-fold) within the syringe barrel by filling the remaining capacity of the syringe with nitrogen. To achieve a 100-fold dilution all but 250 ul of the 10-fold diluted sample volume was expelled and the syringe was again filled to its nominal capacity (2.5 ml) with nitrogen. A 1.0 ml volume of the diluted sample was injected on column for quantification of the headspace vinyl chloride levels.

Analysis of gas phase samples for vinyl chloride was performed using an SRI Model 8610 gas chromatograph with a PID (Photo Ionization Detector). Chromatography was performed on a Restek megabore column (MXT-Volatiles), 30 meter x 0.53 mm ID x 3.0 um film. Separation and elution of constituents was performed under an isothermal temperature program at $60^0$ C for 5 minute followed by a thermal gradient to $220^0$ C at $10^0$ C per minute to purge other constituents from the column. A gas phase calibration standard of chlorinated ethene's was used to develop a five-point calibration curve for vinyl chloride. The calibration mixture was composed of the following constituents in mole percentage:

- Vinyl chloride 100 ppm
- Cis-1,2-dichloroethylene 103 ppm
- Trichloroethylene 98.5 ppm
- Perchloroethylene 101 ppm

# SECTION 3: PRELIMINARY MICROCOSM SCREENING

Aerobic serum bottle microcosm studies are difficult to implement and can suffer from oxygen limitation within the systems, especially with moderate to high organic carbon or contaminant loading. Furthermore, levels of carbon dioxide can increase to inhibitory levels effecting the over biodegradative performance. Repeated flushing of microcosm headspace with air or oxygen can lead to the degeneration of the microcosm seals and result in the loss of volatile contaminant mass. Therefore as a prerequisite to testing, initial screening experiments are performed to range find the optimum parameters for final test system design. Samples from both site areas were subjected to this initial screening procedure.

## Experimental Design

The COC that are the subject of these microcosm tests are all known to be aerobically biodegraded, and are capable of serving as the sole source of carbon and energy to support the growth and activity of indigenous microorganisms. Therefore, these screening tests focused on two treatments to evaluate the biodegradation potential for each location as summarized below.

***Sterile Controls.***

Microcosms were prepared with sterilized soil and groundwater. Soil was sterilized (120$^0$ C @ 15 psi for 60 minutes) by autoclaving the microcosm bottles after sediment addition and crimp sealing them with Teflon lined septa. The bottles were immersed in a water bath and sterilized for three, one hour cycles with intermediate time intervals of three days. This allowed for complete cooling of the sediments and the opportunity for germination of microbial spore formers. Groundwater was steam sterilized by autoclaving @ 120$^0$ C and 15 psi for 30 minutes. Sterile groundwater was aseptically transferred to the sterile sediment serum bottles. Groundwater was supplemented with standard solutions of the specific COC to achieve levels that were within an order of magnitude of the groundwater at the individual site locations.

***Aerobic Unamended.***

Unamended microcosms represent the baseline condition prevailing at the site locations under evaluation. They serve as a baseline reference for comparing the level of activity that may be expected in the absence of supplemental treatments with those attainable with biological enhancements (i.e., oxygen, inorganic nutrients, organic supplements). No additions or supplements were made to these microcosms initially. However, when redox indicator resazzurin went colorless indicating available oxygen had been depleted, air was injected to replenish the oxygen supply.

***Oxygen Amended.***

Oxygen supplementation is demonstrated to be a potential remedial alternative to enhance aerobic biodegradative processes for a wide range of organic pollutant impacted plumes. Air sparging and peroxide addition to groundwater are two methods available for increasing dissolved oxygen levels and aerobic activity. Oxygen amended microcosm test were performed to determine the extent that biological activity could be enhanced with this treatment alternative. Pure oxygen gas was injected into the headspace of designated microcosms to provide an enriched oxygen environment.

Screening studies were conducted in 160 ml glass serum bottles to obtain an initial estimate of the extent of biological activity present in the site materials and to ascertain difficulties that may be encountered during testing. For these screening tests, sediment and groundwater from both site areas were added to test systems at a ratio of 100 grams sediment to 100 ml of groundwater. This is a ratio that was estimated would provide reasonable results based on the limits of the laboratory analytical testing methods. In addition, higher sediment to groundwater ratios is a better approximation of the actual in situ condition. Homogenized sediment sample was weighed into tarred serum bottles and the final sediment weights recorded for each bottle.

The reduced form of the redox indicator dye, resazzurin, was added to groundwater as a means of visibly discerning that microcosms did not go anoxic during testing. Groundwater was prepared for the active microcosms by sparging the water with air at $15^0$ C to achieve a dissolved oxygen level of approximately 8.0 – 9.0 mg/L.

For the vinyl chloride microcosms, aerated 34-OB12 groundwater was transferred to a 2.0 - liter Tedlar bag and 3.0 ml of gaseous vinyl chloride was injected to achieve a target liquid concentration of 4,150 ug/L. The groundwater was allowed to equilibrate with the vinyl chloride for a period of 24 hours at $10^0$ C prior to the contents being taken up into a 1.0 liter gastight syringe. Vinyl chloride microcosm bottles previously filled with sediment were then supplemented with 100 mls of groundwater delivered from the syringe and immediately capped and sealed. Sterile groundwater was not aerated but was supplemented with vinyl chloride as described. All apparatus used for preparing the sterile groundwater was previously sterilized.

PAH microcosms received aerated 33-MW6FP groundwater that had been aerated as previously described. Supplementation of groundwater with a spike solution of target PAH constituents was not performed. Once PAH microcosms had been assembled they were equilibrated for 5 days at $10^0$ C prior to beginning the test.

A headspace volume representing 10 to 15% of the total microcosm volume (i.e., 16-20 ml) was available as a means of supplying oxygen as well as a means of sampling for volatile and semi-volatile constituents to measure the extent of biodegradation. The microcosm bottles were crimp sealed with Teflon-lined butyl rubber septa that allowed for removal of liquid and/or headspace samples with minimal potential for loss of volatile constituents during these initial screening tests.

The headspace of oxygen-amended microcosms was supplemented with 10 milliliters of pure oxygen (14.28 mg) using a gastight syringe to inject the gas through the microcosm septum. To maintain similar pressures within all microcosms, aerobic (unamended) microcosms were injected with 10 milliliters of air while sterile microcosms were injected with 10 mls of nitrogen gas.

Microcosms were incubated at $20^0$ C in a constant temperature incubator. Sampling and analysis was performed over a period of 21-days at designated time intervals (0, 1, 3 5, 7, 10, 14 and 21 days).

## Results & Discussion

***Aerobic PAH Biodegradation Microcosms.***

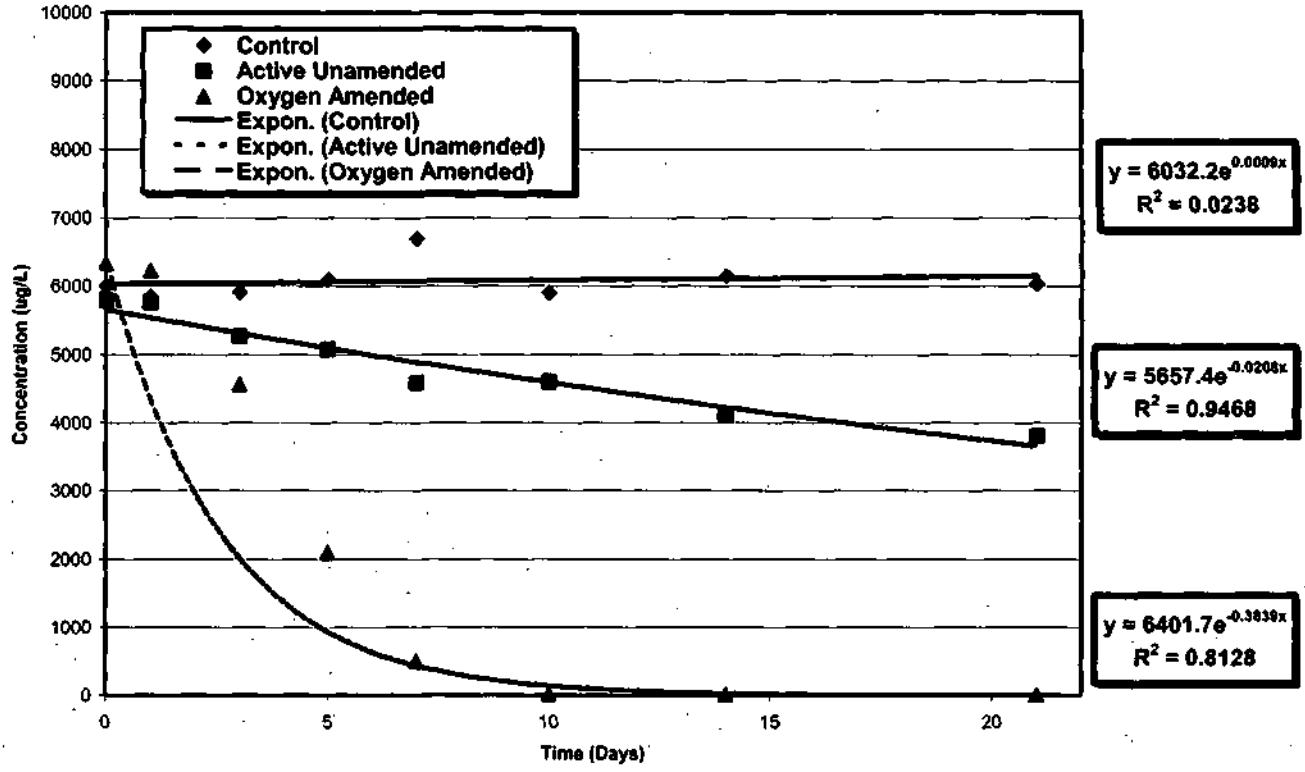
Active degradation of the target PAH constituents was observed in the both the aerobic and the oxygen enhanced microcosms (Figure 1). In the Active Unamended microcosms, 34.48% of the total 2- and 3-ring PAH present were degraded over the 21 day test duration. The rate of PAH degradation had a first-order rate constant of -0.021 $day^{-1}$ or a half-life of 33 days ($T_{1/2}$ = 0.693/k). During the test the Active Unamended microcosms went anaerobic within 3 days as determined by the change in the redox indicator dye. On each sampling day 10 milliliters of headspace was exchanged with fresh air in an attempt to simulate the natural oxygen recharge that may occur under natural conditions in situ. However, only slight changes in redox indicator where observed and for very short durations (i.e. 24 hours).

As indicated by the trend analysis in Figure 1, the oxygen supplemented microcosms exhibited higher rates of biodegradation yielding higher PAH degradation efficiencies (99.84%). The first order aqueous phase rate constant for PAH degradation calculated from this data is equivalent to - 0.3839 $day^{-1}$ or a half-life of 1.8 days. Virtually all of the aqueous phase PAH was consumed within 10 days of test initiation. However, when sediment samples where analyzed at the final time point a significant residual amount of 2- & 3-ring PAH remained (14.8 ug/g). This residual accounts for approximately 58% of the initial 2- & 3-ring PAH sediment load. By day 5 of these tests the redox indicator began to go colorless in the oxygen amended microcosms indicating oxygen was becoming limiting. At this time 10 milliliters of the microcosms headspace was exchanged with oxygen gas.

These results suggest that; (1) PAH degradation in aerobic waters may be quite rapid, and (2) the rate of PAH biodegradation in the aqueous phase exceeds the desorption rate of these compounds from the sediments, and that supplementation of groundwater with oxygen may rapidly treat dissolved phase constituents. Several factors unique to the microcosms may contribute to these results. First, oxygen delivery efficiency is going to be much higher than will be observed under field conditions. Second, microcosms are static batch tests and therefore the levels of microorganisms that may develop in the aqueous phase will exceed that of in situ groundwater. Therefore, the higher biomass loads in the

microcosms create a bias in rates and efficiencies. Third, even though these rates are high it appears that oxygen limitation may still have been a factor during these tests.

**Figure 1: PAH (Σ 2-Ring to 3-Ring) Degradation Trends in Aerobic Microcosm Screening Test**



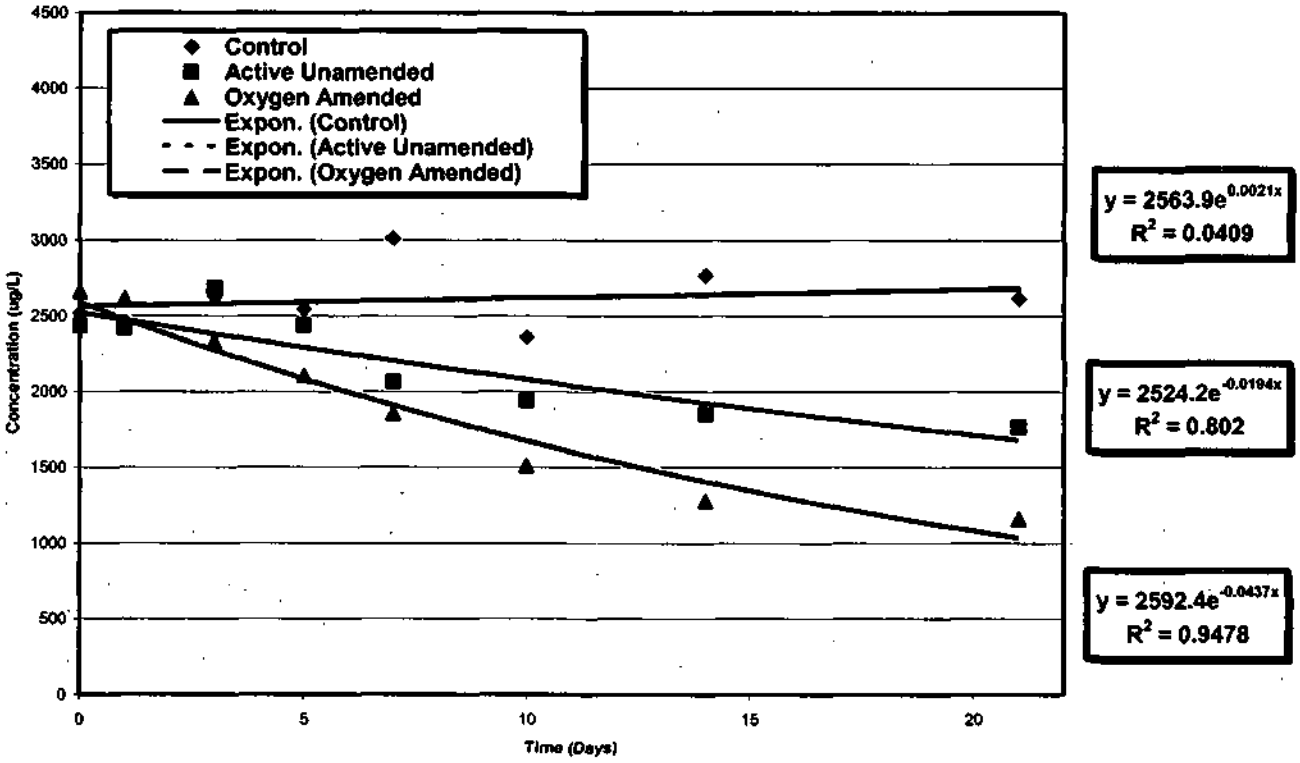

While these results are encouraging they do not provide an indication of the rate at which the sorbed contaminant mass will biodegrade. Aerobic serum microcosm bottle tests are not an effective system for evaluating aerobic biodegradation of the sorbed contaminant mass. A optimum system for tracking the degradation of the sorbed contaminant mass under aerobic conditions will allow for a larger treatment volume that can accommodate repeated sediment sampling. In addition the system should allow for oxygen delivery on demand so that the system never becomes oxygen limited and should allow for removal of carbon dioxide so that inhibitory levels do not develop.

### *Vinyl Chloride Microcosms*

Vinyl chloride levels in the Sterile Control microcosms remained relatively constant throughout the 21 day duration of the test (Figure 2). This result suggests that the

sediments and groundwater had been effectively sterilized by the sterilization procedure employed. These results also suggest that the system integrity was more than adequate in preventing the escape of fugitive volatile emissions from microcosms during the test program. Furthermore, it demonstrates that repeated headspace sampling had little if any impact on the vinyl chloride levels results through the duration of the testing program.

**Figure 2. Vinyl Chloride Degradation Trends in Aerobic Microcosm Screening Test**




Onset of vinyl chloride biodegradation in Active Unamended microcosms occurred over a seven-day time-period suggesting that acclimation of the microbial populations was occurring (Figure 2). Acclimation is the period required for induction of biodegradative enzymes and/or the growth of microbial populations to sufficient density for significant contaminant mass removal to be discernable. Over the duration of the test 22.5% pf the initial vinyl chloride present was degraded. The first order aqueous phase rate constant for this unamended activity is -0.0184 $day^{-1}$ and was derived from the best-fit trend line for the data series (Figure 2). Over the duration of this test it was observed that the redox indicator dye began to go colorless, indicating oxygen depletion, between day 14 and 21 of the test

program. Based on this data, the vinyl chloride half-life under oxygen limiting conditions is on the order of 37.7 days.

In the Oxygen Amended microcosms, the acclimation appeared to be considerably reduced to three-days before onset of significant biodegradation was observed. During the 21 day test period 56.4% of the vinyl chloride mass appears to have been biodegraded. The first order aqueous phase rate constant for this oxygen-enhanced process is -0.0487 $day^{-1}$ and is approximately 2.5 times faster than the Active Unamended rate. The half-life of for vinyl chloride in these oxygen-enhanced microcosms is approximately 14.2 days. During the test, the redox indicator dye went colorless in the Active Control series. The oxygen amended microcosms had a significant change in the intensity of the color and where supplements with 5.0 mls of additional oxygen on day 5 of testing, after which the color appeared to diminish slightly.

These results suggest that a robust vinyl chloride biodegradation potential exists in the impacted site materials from this area of the plume. It further suggests that the aerobic biodegradation potential is stimulated and enhanced by the addition of supplemental oxygen to the system.

## Conclusions

The screening test results provided a quick insight into the biodegradative potential of the site sediments, while providing an assessment of the feasibility of the test systems to support the desired data collection necessary to accurately quantify this biodegradative potential. Based on the screening results Pelorus Environmental & Biotechnology Corporation modified testing protocols to accommodate the constraints imposed by the nature of the site materials.

### *Aerobic PAH Biodegradation*

Results of the PAH screening test suggest that serum microcosm bottle tests will not be an adequate system for assessing the aerobic bioremediation potential of the site sediments and groundwater due to the high oxygen demand of the sediments. Furthermore, assessment of the bioremediation potential, using measures of the aqueous phase or even headspace vapor phase will likely lead to a gross overestimate of the biodegradation

potential. This conclusion is based on the fact that, the aqueous phase hydrocarbon levels are rapidly depleted by biological activity. Based on the results of the screening test it was determined that a respirometer test system would provide a number of advantages over microcosm serum bottles for accurately assessing PAH biodegradation potential. Therefore, further testing of the DNAPL sediments was performed using an N-Con Systems, Comput-Ox Respirometer. These tests are the subject of Section 4 of this report.

***Vinyl Chloride Biodegradation***

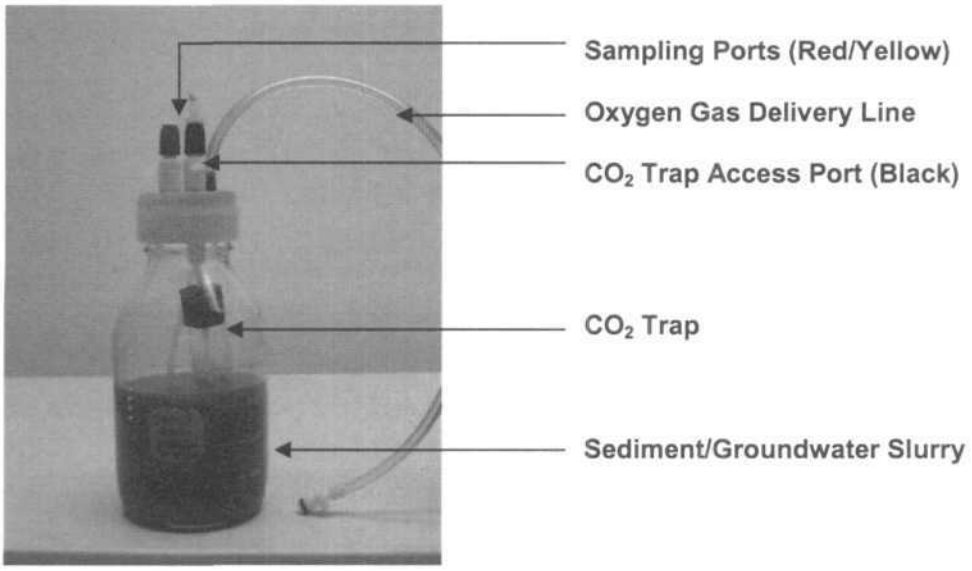
The serum bottle microcosms were an adequate system design for assessing aerobic bioremediation potential of impacted site materials. The nature of the substrate makes it conducive to vapor phase sampling and analysis of microcosm headspace to evaluate trends in contaminant biodegradation. One problem encountered during the screening test is related to the vinyl chloride gas purchased for supplementing microcosms to achieve levels commensurate with site conditions. In order to stabilize or preserve vinyl chloride low levels of phenol (5%) are added to the gas cylinders. It is well documented that certain phenol degrading microorganisms will cooxidize vinyl chloride. Therefore, the results observed during the screening test may be compromised due to the presence of low levels of phenol (~125 ug/L).

Pelorus Environmental & Biotechnology Corporation developed a process to remove phenol from the vinyl chloride gas used to supplement microcosm groundwater. This process involves running the gas through a silica gel cartridge to adsorb the phenol from the gas stream prior to addition to the test systems. The treated gas is prepared and stored in a 1.0 liter Tedlar bag immediately prior to use. Analysis of the treated vinyl chloride gas by GCMS and by GCFID indicated that phenol had been effectively removed from the gaseous vinyl chloride substrate.

To obtain a more legitimate assessment of the vinyl chloride bioremediation potential, the decision was made to run serum-bottle microcosm tests scaled to a larger reactor size and rerun with the processed vinyl chloride substrate. In addition, to minimize the rate of oxygen depletion in microcosms during the test, a lower soil to groundwater ratio should be evaluated. Fresh groundwater was requested and supplied from the site by the Forrester Group. The results of these activities are the subject of Section 5 of this report.

# SECTION 4: RESPIROMETER TESTS FOR ASSESSMENT OF AEROBIC PAH BIODEGRADATION

Based on the results obtained from the screening tests it was determined that the evaluation of PAH biodegradation potential would be best performed in a laboratory respirometer system. Pictures of the respirometer reactor and a description of the components are shown in below.




There are a number of advantages associated with the use of this type of system. First, as with serum-bottle microcosms the test matrix materials are completely sealed within the reactor so that fugitive emissions can be limited. The reactors have a 400-milliliter capacity meaning that larger volumes of test materials can be used thus reducing replicate variability resulting from matrix heterogeneity. Sampling ports on the reactor cap allow for removal of headspace samples and aqueous phase samples without opening the reactor. Once the contents have been added to the reactor it is placed in a temperature controlled water bath and the flexible tubing connected to an oxygen delivery manifold. Under computer control, metered pulses of oxygen are delivered to the reactor as needed. The delivery of oxygen is triggered by a pressure drop within the reactor that results as carbon dioxide is scrubbed from the gas phase in a sodium hydroxide trap (Inner reservoir). Spent alkaline trapping solution can be removed and replenished from an access valve in the reactor cap. The water bath can hold up to twelve reactors simultaneously and each reactor

compartment is equipped with a magnetic stirrer for mixing the reactor contents during testing. With sediment "slurry" systems magnetic stir bars are not effective for mixing and therefore the reactor contents are normally mixed by manually agitating the reactors every 8 hours. The computer software continuously records the data and plots the oxygen consumption throughout the duration of the test.

## Experimental Design

Two respirometer tests were performed with the DNAPL area site materials. This decision was driven by the fact that after homogenization of site samples the 2- and 3-ring PAH profiles tested lower (Table 1, Section 1) than what site characterization data suggested should be present (Table 4 below). Therefore, one test was performed with sediments as received and in the other test sediments were spiked with a cocktail of 2- and 3-ring PAH to achieve target levels more consistent with the site characterization data. The first test was designated *Respirometer: PAH Test # 2* to distinguish it from the screening test (Test #1). Respirometer testing on PAH spiked sediments is designated as *Respirometer: PAH Test #3*

**Table 4.** Summary of Site Characterization Data for 2 and 3-Ring PAH Concentrations in Sediment and Groundwater.

| **Pond Surface Water & Initial Groundwater Data** | | | **Sediment Boring Data** | | |
|---|---|---|---|---|---|
| ***Parameter Name*** | ***Units*** | ***33-MW6FP*** | ***Units*** | ***33-B16 (16 - 17)*** | ***33-B25 (17 - 18)*** |
| ***2-Methylnaphthalene*** | ***µg/L*** | ***28*** | ***mg/Kg*** | ***100*** | ***1300*** |
| Acenaphthene | µg/L | **130** | mg/Kg | **79** | **530** |
| Acenaphthylene | µg/L | **6** | mg/Kg | **4.2** | **55** |
| Anthracene | µg/L | **18** | mg/Kg | **48** | **350** |
| Fluorene | µg/L | **52** | mg/Kg | **32** | **340** |
| ***Naphthalene*** | ***µg/L*** | ***140*** | ***mg/Kg*** | ***77*** | ***1800*** |
| ***Phenanthrene*** | ***µg/L*** | ***84*** | ***mg/Kg*** | ***220*** | ***1400*** |
| Pyrene | µg/L | **11** | mg/Kg | **150** | **1100** |

***Respirometer: PAH Test #2***

A series of twelve respirometer reactors were established to evaluate four conditions in triplicate. Table 5 summarizes the testing matrix and describes in general terms the treatments evaluated. With the exception of the Active Controls, all reactors were supplied oxygen on demand by connecting each to the respirometer manifold. The active controls were sealed with a solid screw cap and rubber gasket seal that prevented emissions of vapors or diffusion of air into the reactor headspace.

**Table 5.** Respirometer: PAH Test #2 Treatment Matrix Description

| TREATMENT | DESIGNATION | DESCRIPTION |
|---|---|---|
| Sterile Control | OPS-A<br>OPS-B<br>OPS-C | Autoclaved sediments & groundwater.<br>Oxygen supplied on demand.<br>No amendments. |
| Active Control | OPA-A<br>OPA-B<br>OPA-C | Site sediments & groundwater.<br>Headspace refreshed at designated<br>sampling intervals. No amendments |
| Oxygen Amended | OPO-A<br>OPO-B<br>OPO-C | Site sediments & groundwater.<br>Oxygen supplied on demand. |
| Oxygen/Nutrient Amended | OPON-A<br>OPON-B<br>OPON-C | Site sediments & groundwater.<br>Inorganic nutrients (N,P, S, K & Fe)<br>Oxygen supplied on demand. |

Each respirometer reactor received approximately 175 grams of homogenized DNAPL area sediments. Groundwater that had been collected from 33-MW6FP was supplemented with the reduced form of the redox indicator resazzurin, a dye that turns pink in the presence of oxygen, and then divided into two batches. One batch was aerated for fours to achieve dissolved oxygen levels in the range of 7.3 to 8.5 mg/L. Two hundred milliliters was then dispensed into each of the reactors from the Sterile Control, Active Control, and Oxygen amended series. The second batch of groundwater was similarly aerated except that an inorganic nutrient cocktail was added to provide the following final concentrations of inorganic salts:

- $NO_3$ 25 mg/L
- $PO_4$ 100 mg/L
- $SO_4$ 50 mg/L
- Fe, K, Mg 5.0 mg/L each

Two hundred milliliters of the aerated and nutrient supplemented groundwater was then added to each reactor of the Oxygen/Nutrient series. The reactors were then placed in the respirometer water bath at $20^0$ C and attached to the oxygen delivery manifold. Prior to initiation of testing the reactors in the Sterile Control series were sealed and submerged in a tub of water and then autoclaved for 3-two hour cycles at $125^0$ C @ 15 psi. The reactors were allowed to sit for period of 24 hours between cycles to allow for the germination of bacterial spore producers.

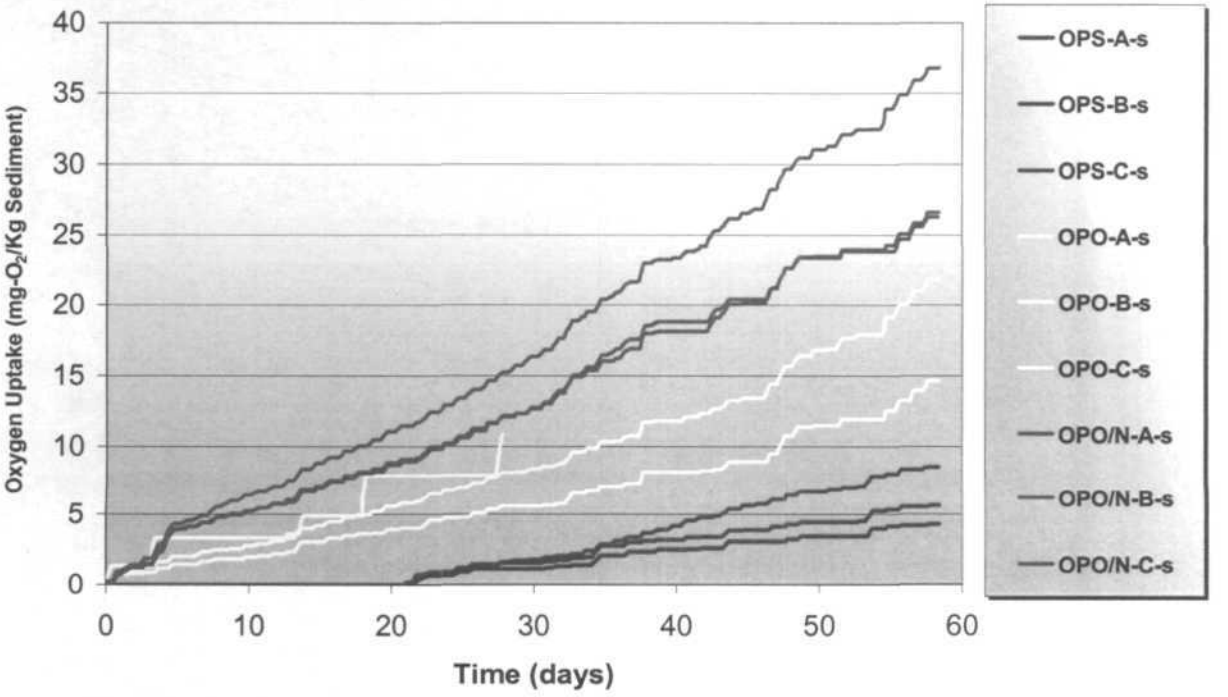
Reactors were sampled approximately every two weeks based on the overall biodegradative performance. The actual sampling events occurred at; $T_0$, $T_{13}$, $T_{28}$, $T_{44}$, and $T_{60}$ days. The sampling procedure involved allowing the sediments to settle and decanting the aqueous phase into a 20 ml graduated cylinder. Two duplicate 40 ml samples were collected in sulfuric acid preserved VOA vials and archived at $10^0$ C. Approximately 10 grams of sediment were removed and placed in 40 ml sample extraction vials. Once the sediment samples were removed the liquid phase was replenished with 80 mls of fresh groundwater, the reactors sealed, mixed and placed back into service on the respirometer. (*Note: The liquid phase concentrations of naphthalene, 2-methylnaphthalene and phenanthrene were essentially non detectable by the $T_{13}$ sampling point. Thereafter aqueous phase sampling was abandoned as being of no value*). A summary of the all the analytical data results for Respirometer: PAH Test #2 is presented in Table A-1 of Appendix A.

## Results & Discussion

Oxygen uptake profiles for each of the treatment series, (Sterile Control/OPS, Oxygen Amended/OPO, Oxygen/Nutrient Amended/OPON) is presented in Figure 3. The overall trends in oxygen uptake suggest that the oxygen and nutrient supplemented treatment (OPON-Series) was the most biologically active, followed by the oxygen supplemented (OPO-Series). Unexpectedly the sterile control (OPS-Series) began to exhibit activity after about 21 days. The results of the sterile may be due to cross contamination during sampling or more likely the germination of spore forming microorganisms that survived the sterilization procedure. The respirometer port for one of the oxygen amended treatments (OPO-A) developed and electrical relay problem and

stopped transmitting data. It did however continue to receive oxygen as the solenoid sensor was still functional.

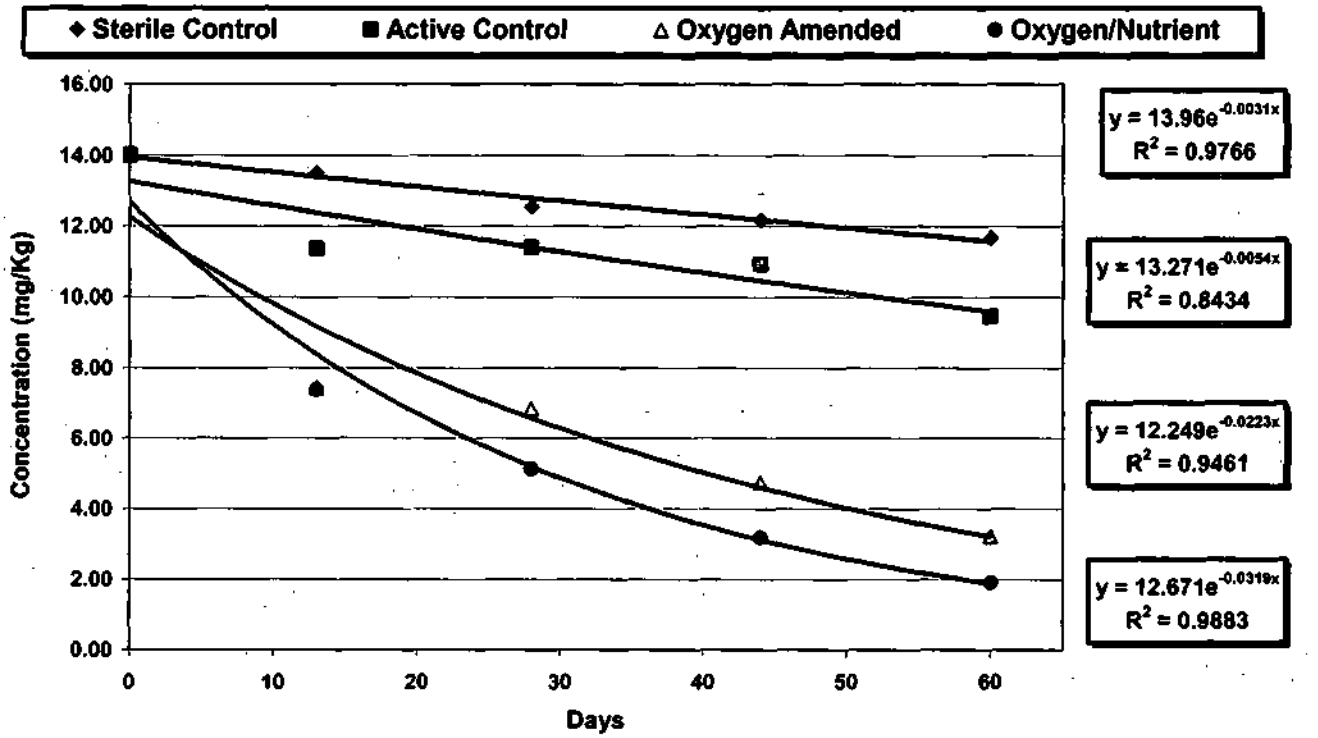
**Figure 3. Oxygen Uptake in Ogden PAH Respirometer #2 Microcosm Study.**




The PAH trends followed the same general trend as observed with the oxygen uptake rates (Figure 4). The highest efficiencies and rates of biodegradation were associated with those reactors receiving the highest oxygen supplements. Total 2- and 3-ring PAH biodegradation efficiencies in the Active Control series were on the order 32.6% with a first-order sediment biodegradation rate of -0.0054 $day^{-1}$. These results suggest that under oxygen limiting natural attenuation conditions in situ, the total PAH's may have a half-life that is on the order of 128 days. PAH biodegradation rates in the Oxygen Amended series are five-fold higher than the Active series, with a first-order sediment biodegradation rate for total PAH biodegradation of -0.0223 $day^{-1}$ or half-life of 31 days assuming that oxygen is not limiting. Inorganic nutrient supplementation along with oxygen amendment contributed to a 30% higher rate of biodegradative activity. The rate constant under these conditions was -0.0319 $days^{-1}$ for total PAH or half-life of 21.7 days. Trends of the target PAH constituents; naphthalene, 2-methylnaphthalene and phenanthrene are illustrated in

Figures 4-a to 4-c (Appendix B). Oxygen supplementation significantly stimulated the rates of biodegradation of each of these target constituents over the natural rates under oxygen limiting conditions (Active Control).

**Figure 4. Total (2 & 3-Ring) PAH Biodegradation During Respirometer: PAH Test #2**




Phenanthrene trends offered interesting insights into the dynamics and selectivity of substrate utilization. Active Control microcosms had little if any phenanthrene degradation based on a comparison with the Sterile Control series (Figure 4-c, Appendix B). However, naphthalene and methylnaphthalene both biodegraded in the oxygen, and nutrient supplemented microcosm series. The small differences in rates of biodegradation between the oxygen and oxygen/nutrient amended series may be an indication that mass transfer is the dominant factor at the concentrations of these constituents in the sediment. Table 6 summarizes the first order rates and degradation efficiencies for each of the target PAH constituents in this test. Phenanthrene is the slowest degrading constituent and can therefore be used as the conservative indicator of remediation progress. Under the oxygen limited natural attenuation condition (Active Control) a half-life of 266 days is expected for

phenanthrene biodegradation. With oxygen supplementation the half-life for phenanthrene is 34 days almost an order of magnitude increase in rate.

**Table 6.** Summary of First-order rate constants and biodegradation efficiencies of target PAH constituents during Respirometer: PAH Test #2.

| TREATMENT SERIES | Naphthalene | | Methylnaphthalene | | Phenanthrene | |
|---|---|---|---|---|---|---|
| | *Rate ($day^{-1}$)* | Efficiency % | *Rate ($day^{-1}$)* | Efficiency % | *Rate ($day^{-1}$)* | Efficiency % |
| ***Sterile Control*** | *-0.0039* | 19.1% | *-0.0036* | 22.4% | *-0.0021* | 13.4% |
| ***Active Control*** | *-0.0086* | 41.1% | *-0.0114* | 55.3% | *-0.0026* | 20.4% |
| ***Oxygen*** | *-0.0236* | 78.6% | *-0.0219* | 74.6% | *-0.0204* | 74.6% |
| ***Oxygen/Nutrient*** | *-0.0367* | 89.5% | *-0.0264* | 83.5% | *-0.0262* | 82.0% |

## Experimental Design

### *Respirometer: PAH Test #3*

Three respirometer reactors were established to evaluate the effect of higher target PAH loadings on aquifer sediments. Table 7 summarizes the testing matrix and describes in general terms the treatments evaluated. With the exception of the Active Controls, reactors were supplied oxygen on demand by connecting each to the respirometer manifold. The active controls were sealed with a solid screw cap and rubber gasket seal that prevented emissions of vapors or diffusion of air into the reactor headspace.

**Table 7.** Respirometer: PAH Test #3 Treatment Matrix Description

| TREATMENT | DESIGNATION | DESCRIPTION |
|---|---|---|
| Sterile Control | OPS | Autoclaved sediments & groundwater. Oxygen supplied on demand. No amendments. |
| Active Control | OPA | Site sediments & groundwater. Headspace refreshed at designated sampling intervals. No amendments. |
| Oxygen/Nutrient Amended | OPON | Site sediments & groundwater. Inorganic nutrients (N,P, S, K & Fe) Oxygen supplied on demand. |

Each respirometer reactor received approximately 175 grams of homogenized DNAPL area sediments that had been spiked with a PAH cocktail to achieve the following target values:

- Naphthalene 40 mg/Kg
- 2-Methylnaphthalene 50 mg/kg
- Phenanthrene 100 mg/Kg

The cocktail was prepared in diethyl ether and applied to homogenized sediment using a spray mister. Sediment was weighed into a large aluminum mixing bowl and the ether solution sprayed onto the surface of the soil as it was being manually mixed. The ether rapidly evaporates and generates a fine particulate mist that coats the soil. After the designated amount of cocktail had been applied and mixed into the sediments they were allowed to weather for a period of 30 days under refrigeration prior to testing.

Groundwater was prepared as described above for Respirometer: PAH Test #2. Two hundred milliliters was then dispensed into each of the reactors; the Sterile Control, and Active Control. The Oxygen/Nutrient amended reactor was supplemented with groundwater containing the inorganic nutrient cocktail to achieve the following final solution concentrations:

- $NO_3$ 25 mg/L
- $PO_4$ 100 mg/L
- $SO_4$ 50 mg/L
- Fe, K, Mg 5.0 mg/L each

The decision to test only the oxygen/nutrient amendment against the active control in this test was due primarily to the higher loadings of PAH that warranted nutrient supplementation, and the fact that in Respirometer: PAH Test #2, nutrients provided a marginal (10 to 14 %) increase in extent of biodegradation but a significant (20-55%) rate enhancement. Therefore, the effects of higher PAH loading could be isolated from oxygen and nutrient limitation by comparing results of the oxygen/nutrient supplemented treatments between Test #2 and Test #3.

After the reactors had been filled and baseline sampled they were placed in the respirometer water bath at $20^0$ C and attached to the oxygen delivery manifold. Prior to initiation of testing the Sterile Control reactor was sealed and submerged in a tub of water and then autoclaved for 3-two hour cycles at $125^0$ C @ 15 psi. The reactor was allowed to

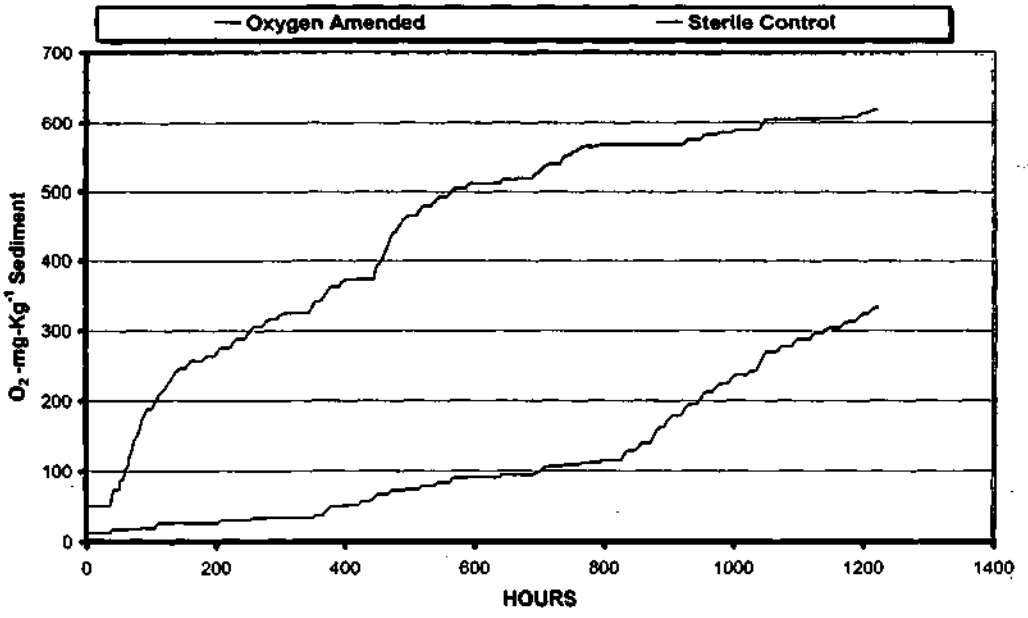
sit for period of 24 hours between cycles to allow for the germination of bacterial spore producers.

Reactors were sampled approximately every two weeks based on the overall biodegradative performance. The actual sampling events occurred at; $T_0$, $T_{15}$, $T_{29}$, and $T_{41}$, days. The sampling and analysis procedure was identical to that described above for Respirometer Test #3. A summary of the all the analytical data results for Respirometer: PAH Test #2 is presented in Table A-2 of Appendix A.

## Results & Discussion

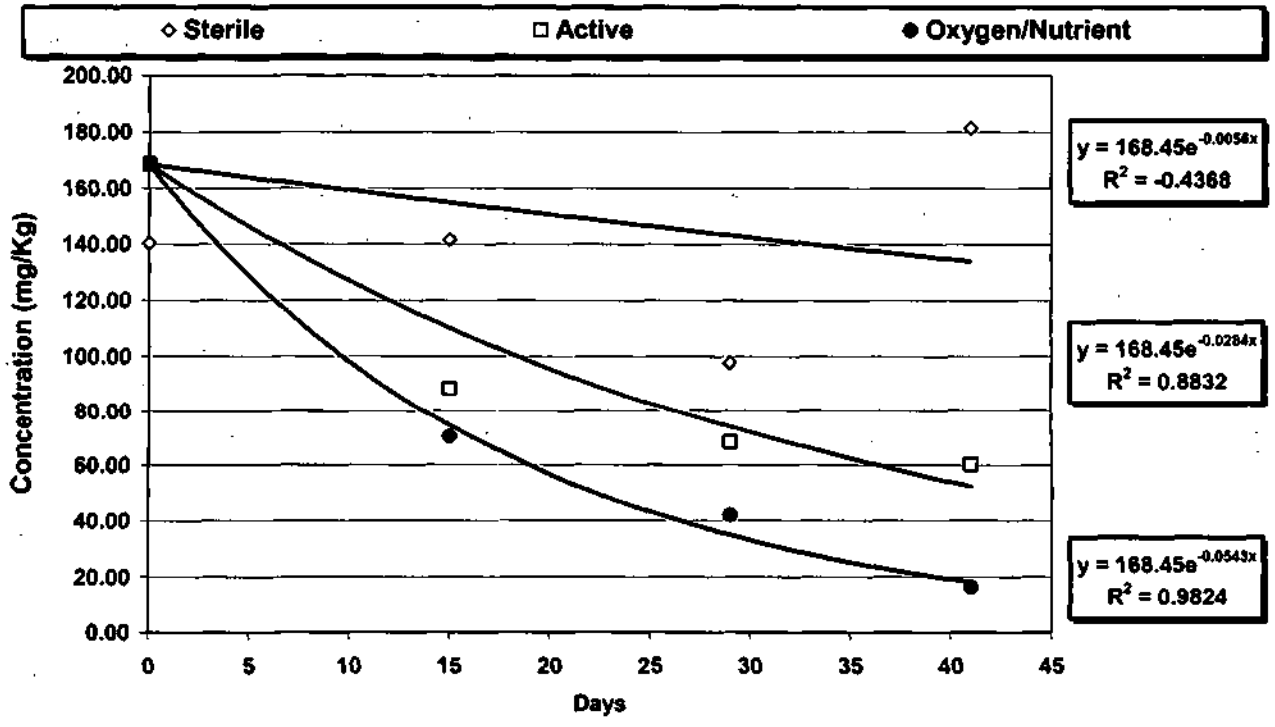
Oxygen uptake rates were significantly higher in than the previous test because of the higher concentrations of 2-ring and 3-ring PAH spiked into the test sediments. As previously observed the Sterile Control begins to become active, in this case after about 15 days (360 hours) and after approximately 35 days (850 hours) is highly active (Figure 5). Microbial analysis of an aqueous phase sample from the sterile control by serial dilution plating on nutrient agar showed the presence of microbial population density of 4 x $10^6$ colony forming units per ml. Based on colony morphology and microscopic examination of cells from individual colonies it was determined that a high percentage (~70%) were Bacillus and Actinomycete type organisms both of which are spore forming organisms.

**Figure 5: Oxygen Uptake in Ogden PAH Respirometer: PAH Test #3**



Analysis of sediment samples for the target 2-ring and 3-ring PAH throughout the duration of the test provided data indicating that higher rates of biodegradation were occurring in the Oxygen/nutrient amended system as compared to the Active system receiving no amendments (Figure 6). Under the oxygen limited or natural attenuation condition (i.e., Active Control), the observed first-order sediment biodegradation rate constant for total target PAH (i.e., the sum of 2-ring and 3-ring PAH) biodegradation was -0.0284 $day^{-1}$ with an overall treatment efficiency 64.2%. The oxygen plus nutrient amended treatment resulted in 91% increase the first-order sediment biodegradation rate (-0.0543 $day^{-1}$) over the oxygen limited natural rate (Active Control). Because of this enhancement of biodegradative activity, the nominal treatment efficiency for total PAH increased to 90.4% and the half-life went from 24 days to approximately 13 days.

**Figure 6. PAH (2- & 3- Ring) Biodegradation Trends During Respirometer: PAH Test #3**




Trends of the individual target PAH constituents; naphthalene, 2-methylnaphthalene and phenanthrene are illustrated in Figures 6-a to 6-c (Appendix C). Oxygen supplementation significantly stimulated the rates of biodegradation of each of these target

constituents over the natural rates under oxygen limiting conditions (Active Control). A summary of the first order rate constants and biodegradation efficiencies for the target PAH's, naphthalene, 2-methylnaphthalene and phenanthrene are presented below in Table 8. As was observed previously, phenanthrene is the target PAH that degrades the slowest and therefore represents the rate limiting constituent in a potential enhanced bioremediation program.

The net effect of the higher PAH loading on sediments is that the enhanced rates of biodegradation are 1.5 to 3-fold higher than that observed at a 10-fold lower initial PAH concentration as in Respirometer: PAH Test #2. In the Active Control or oxygen-limited situation, the effects of a 10-fold higher initial PAH level are more pronounced with the first-order rate constants ranging from 2 to 8-fold depending on the PAH. These results are consistent with basic principles of enzyme or bacterial reaction kinetics, where the rate of the reaction is a function of the rate limiting substrate concentration.

**Table 8.** Summary of First-order rate constants and biodegradation efficiencies of target PAH constituents during Respirometer: PAH Test #3.

| TREATMENT SERIES | Naphthalene | | Methylnaphthalene | | Phenanthrene | |
|---|---|---|---|---|---|---|
| | *Rate ($day^{-1}$)* | Efficiency % | *Rate ($day^{-1}$)* | Efficiency % | *Rate ($day^{-1}$)* | Efficiency % |
| ***Sterile Control*** | *-0.0002* | 0 % | *-0.0007* | 0 % | *-0.0002* | 0 % |
| ***Active Control*** | *-0.0538* | 81.8% | *-0.0232* | 65.3% | *-0.0211* | 56.4% |
| ***Oxygen/Nutrient*** | *-0.1072* | 97.9% | *-0.0874* | 98.6% | *-0.0383* | 82.2% |

## Conclusions

The DNAPL area of the UPRR Ogden Site has an active population of indigenous microorganisms that possess the capability of biodegrading the target two and three ring PAH constituents impacting groundwater quality flowing towards the 21st Street Pond. These bench scale microcosm and respirometer tests focused on naphthalene, 2-methylnaphthalene, and phenanthrene due to their higher solubility and mobility in groundwater compared to the less water-soluble PAH constituents in diesel and coal tar.

Under oxygen limited conditions these target PAH constituents naturally bioattenuate, the specific mechanisms not defined in the present studies studies. However, it has been documented in the scientific literature, and therefore the precedent established, that both naphthalene and phenanthrene can be anaerobically degraded under sulfate reducing conditions. Based on work performed with monoaromatics hydrocarbons, under oxygen limited conditions it is feasible that some of the available oxygen may be inserted into these PAH molecules to form hydroxylated intermediates (i.e., substituted aromatic acids and phenols). Once formed the resonance structure of aromatic ring system is destabilized, making these molecules easier to mineralize through micro-aerophilic processes such as denitrification and iron reduction or by anaerobic sulfate reduction and methanogenesis.

The presence in site sediments and groundwater, of aerobic microorganisms that can grow on naphthalene, 2-methylnaphthalene and phenanthrene as their sole source of carbon and energy suggests that this site is well acclimated biologically to the target constituents. Substantial biodegradation enhancement of the target PAH constituents by the addition of oxygen to the system suggests that oxygen and perhaps other terminal electron acceptors are limiting the natural attenuation process.

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The presence in site sediments and groundwater, of aerobic microorganisms that can grow on naphthalene, 2-methylnaphthalene and phenanthrene as their sole source of carbon and energy suggests that this site is well acclimated biologically to the target constituents. Substantial biodegradation enhancement of the target PAH constituents by the addition of oxygen to the system suggests that oxygen and perhaps other terminal electron acceptors are limiting the natural attenuation process. Results of these tests therefore support the potential for technologies such air sparging or other oxygen delivery processes as remedial alternatives to facilitate more rapid in situ biotreatment of target PAH constituents.

# SECTION 5: MICROCOSM TESTS FOR THE EVALUATION OF AEROBIC VINYL CHLORIDE BIODEGRADATION

Microbial characterization of site sediments using a selective plate count media and vinyl chloride as the sole carbon and energy source indicated that a population of vinyl chloride degrading organisms may be present. However, these organisms appear to be fastidious as they do not appear to grow well on this substrate under the selective conditions used. Initial serum-bottle microcosm tests also suggested that site sediment and groundwater contained an indigenous population of microorganisms with the metabolic potential to degrade vinyl chloride under aerobic conditions. What could not be determined from the initial screening test was whether the microbial populations aerobically biodegraded vinyl chloride as source of carbon and energy for growth, or whether it was co-oxidatively metabolized due to the presence of a primary growth substrate such as phenol or some other metabolic activator molecule (i.e., aromatic hydrocarbon, methane, propane, butane, ammonia). To confirm that the site areas impacted with vinyl chloride contained microorganisms that could be stimulated to bioremediate dissolved plume impacts by oxygen supplementation, a more thorough microcosm test was performed.

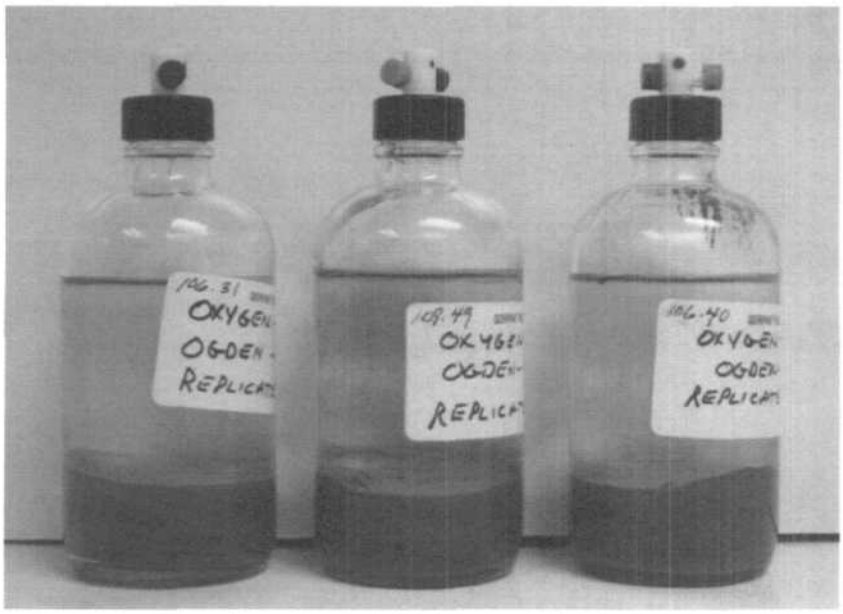
## Experimental Design

The overall experimental design with respect to treatments evaluated were identical to that described in the screening test. Microcosm tests were performed in triplicate for each of the specified treatments, which consisted of:

- **Sterile Control-** Assess abiotic losses
- **Active Control-** Simulates/assesses the natural oxygen limited condition
- **Oxygen Amended-** Simulates potential for oxygen enhanced bioremediation

The only substantive changes to these studies were the size of the serum-bottle microcosms. Tests were scaled-up to be conducted in 200 milliliter screw cap Quorpak bottles. For these screening tests, sediment and groundwater from vinyl chloride impact areas as defined by boring 34-B from which sediment for testing was collected. Approximately, 100 grams of homogenized sediment was added to each microcosm bottle.

Aerated groundwater supplemented with redox indicator dye (i.e., resazzurin) was prepared as previously described and then spiked with the filtered vinyl chloride gas to achieve a solution concentration of approximately 1000 ug/L. One hundred and fifty milliliters of the vinyl chloride spiked groundwater was added to each of the microcosm test bottles. The bottles were sealed with screw cap Mininert valves that allow for repeated sampling of headspace without the destruction of a Teflon lined rubber septum. Below is a picture of the completely assembled microcosm showing the Mininert valve caps and the color of the redox dye supplemented groundwater when dissolved oxygen is present.




The headspace volume represents 10% of the total microcosm volume (i.e., 20 ml) that was left available as a means of supplying oxygen as well as a means of sampling for volatile constituents to analyze the extent of biodegradation.

Sterile microcosms where prepared by autoclaving the bottles after sediment had been added, and then partially screwing the Mininert valves caps on loosely. Groundwater for the sterile microcosms was not aerated or supplemented with the redox indicator dye and was sterilized in a separate container. Once sterile the groundwater was aseptically transferred to a sterile 1.0-liter gastight syringe and supplemented with vinyl chloride gas to achieve the target concentration of 1000 ug/L. The sterile supplemented groundwater was injected in to the sterile sediments and microcosm bottles then sealed with the Mininert valve caps.

The headspace of oxygen-amended microcosms was supplemented with 15 milliliters of pure oxygen using a gastight syringe to inject the gas through the Mininert valve septum. To maintain similar pressures within all microcosms, unamended (Active Control) microcosms were injected with 15 milliliters of air while sterile microcosms were injected with 15 mls of nitrogen gas.

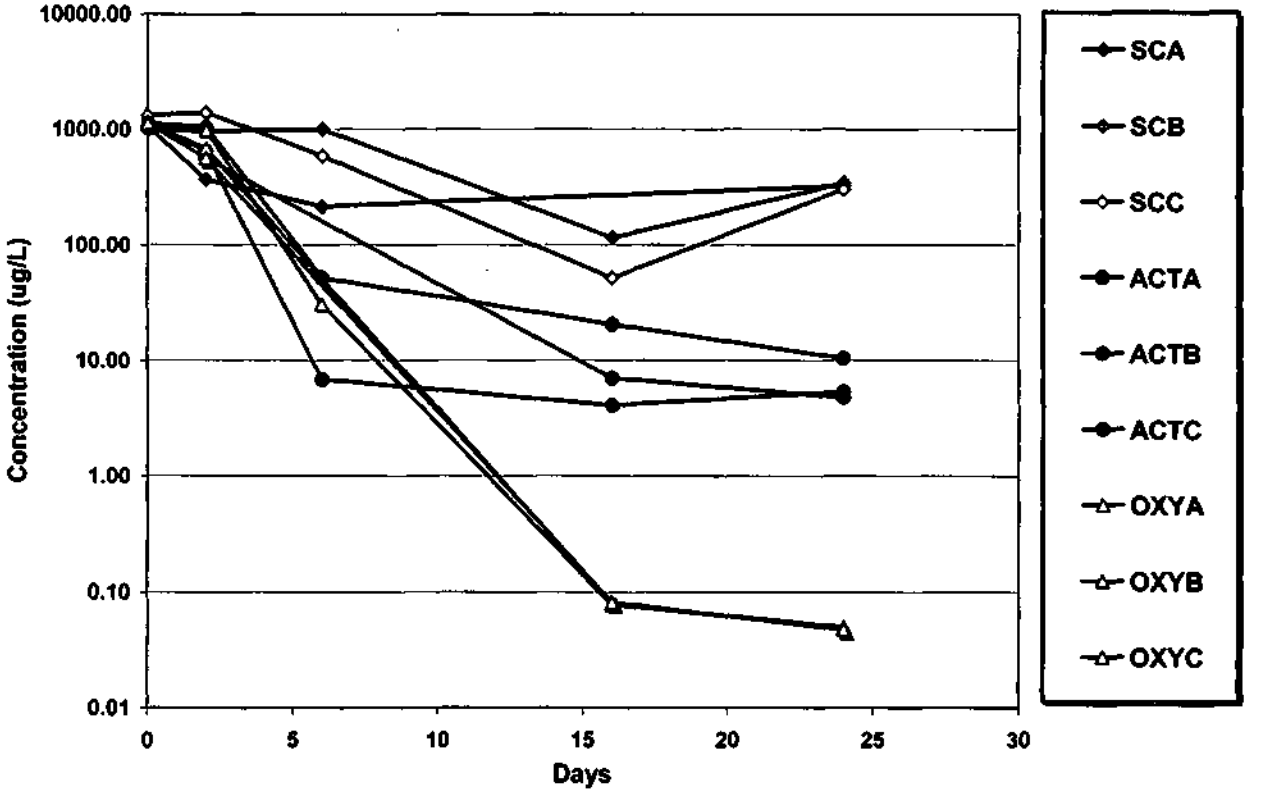
All microcosms were maintained at a constant temperature of $20^0$ C by incubation in a temperature-controlled incubator. Sampling was performed over a period of 24-days at the designated time intervals (0, 2, 6, 16, and 24 days) and vinyl chloride analysis performed immediately upon sampling as previously described.

## Results & Discussion

Visible onset of vinyl chloride biodegradation occurred within three to six days in both the Active Control (ACT-Series) and Oxygen Amended (OXY-Series) in the serum-bottle microcosm tests (Figure 7). These results suggest that an acclimated population of microorganisms is present in site groundwater and sediments that has the capacity to biodegrade vinyl chloride aerobically. Sterile Controls exhibited an overall loss of approximately 70% of the added Vinyl chloride during testing. An evaluation of the microbial population levels indicated that the sterility of the control microcosms was maintained for the duration of the test. Therefore, the observed declines in vinyl chloride levels are not likely to be due to microbial activity. Most of the vinyl chloride loss in the controls occurs within the first 5 days of the test. Another possible explanation is that equilibrium partitioning between the phases within the microcosm occurs during this period resulting in an apparent loss of the vinyl chloride.

Active control microcosms exhibited a 99.4% reduction in vinyl chloride levels most of this occurring during the first five days of testing after which activity appeared to become asymptotic. During this period, the redox indicator dye became very faint to colorless in these microcosms suggesting that oxygen may have become rate limiting. Support for this hypothesis comes in part from the trends observed for vinyl chloride in the Oxygen Amended microcosms where, the vinyl chloride levels continue to decline through the duration of the test reaching an asymptote at the detection limit of 0.05 ug/L.

**Figure 7. Vinyl Chloride Biodegradation Profiles During Microcosm Testing**



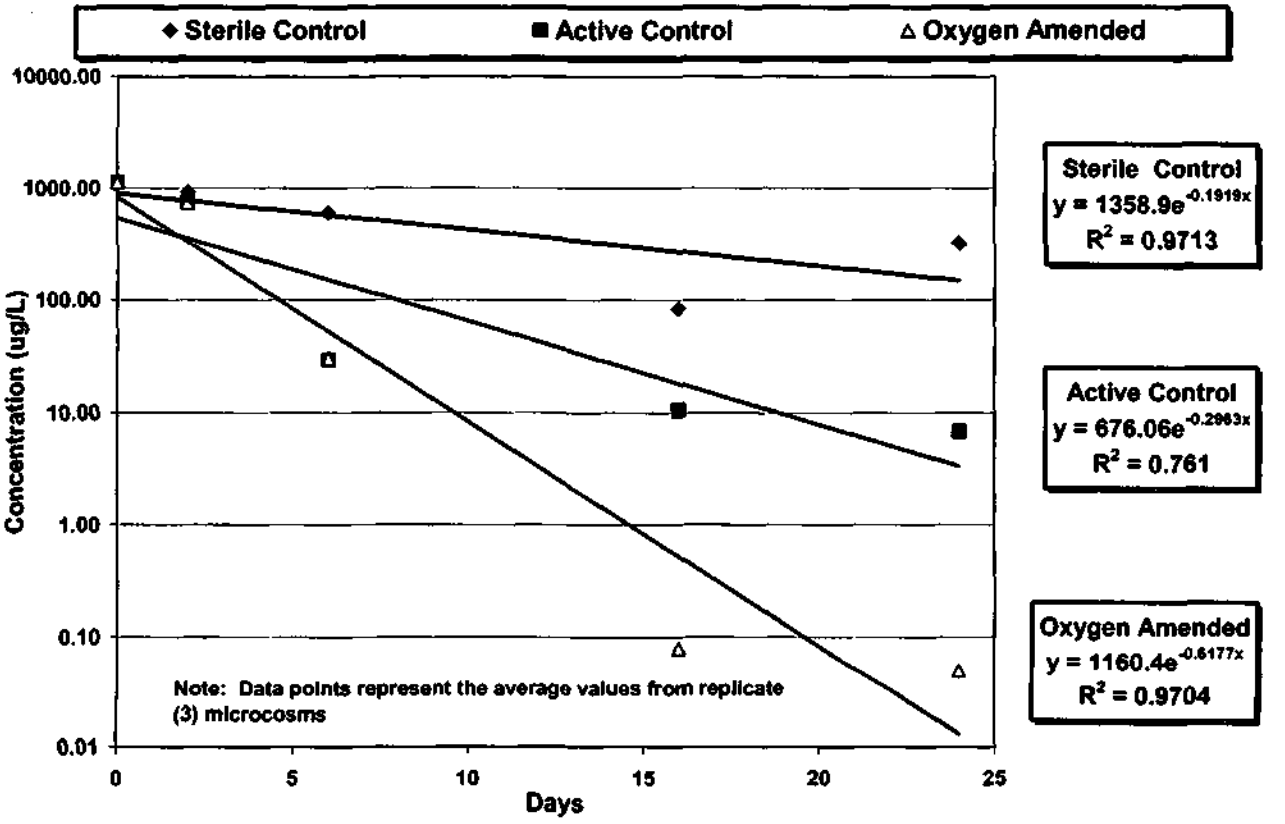

An analysis of these trends was performed by fitting an exponential curve-fit to the microcosm data and determining the first-order rate for the observed reactions (Figure 8). To account for abiotic processes that may be occurring in the systems the first-order rates were corrected for the vinyl chloride loss in the Sterile Controls. Table 9 summarizes the kinetic analysis of the microcosm test data.

**Table 9.** Summary of the first-order kinetic analysis of vinyl chloride data trends during the serum-bottle microcosm testing.

| TREATMENT SERIES | Vinyl Chloride | | |
|---|---|---|---|
| | *Rate ($day^{-1}$)* | *Sterile Control Corrected* | *Half-life (Days)* |
| ***Sterile Control*** | *-0.1919* | | |
| ***Active Control*** | *-0.2963* | -0.1044 | 6.6 |
| ***Oxygen/Nutrient*** | *-0.6177* | -0.4258 | 1.6 |

It is apparent from the microcosm tests that indigenous populations of microorganisms have acclimated to the presence of vinyl chloride and are actively degrading this constituent in the sediments and groundwater. While the individual microorganisms have not been isolated and purified presumptive evidence of their existence was observed from the plating of site samples on selective media with vinyl chloride as the substrate.

**Figure 8. First-order Rate Trends for Vinyl Chloride Biodegradation in Microcosm Tests**



# APPENDIX A

## Microcosm & Respirometer Test Analytical Results

## Table A-1. Summary of Gas Chromatographic/FID Analytical Raw Data for Respirometer Test # 2

| SAMPLE | DAY | SAMPLE DRY WEIGHT (mg) | SAMPLE WET WEIGHT (mg) | [1]EXTRACT CONCENTRATION PAH (ug/ml) | | | TOTAL EXTRACTED MASS PAH (ug) | | | PAH CONCENTRATION D.W. (ug/mg) | | | PAH CONCENTRATION W.W. (ug/mg) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | NP | 2-MNP | PHN | NP | 2-MNP | PHN | NP | 2-MNP | PHN | NP | 2-MNP | PHN |
| | | | | *Sterile Control* | | | *Sterile Control* | | | *Sterile Control* | | | *Sterile Control* | | |
| OPS-A-s-1 | 0 | 11.99 | 15.06 | 58.33 | 83.91 | 25.14 | 87.49 | 125.86 | 37.71 | 7.30 | 8.36 | 5.17 | 5.81 | 8.36 | 2.50 |
| OPS-A-s-2 | 13 | 11.46 | 14.06 | 53.96 | 74.99 | 25.35 | 80.94 | 112.48 | 38.03 | 7.06 | 8.00 | 5.38 | 5.76 | 8.00 | 2.70 |
| OPS-A-s-3 | 28 | 11.4 | 14.1 | 48.76 | 63.20 | 22.19 | 70.15 | 94.80 | 33.28 | 6.15 | 6.72 | 5.41 | 4.97 | 6.72 | 2.36 |
| OPS-A-s-4 | 44 | 12.87 | 15.74 | 49.91 | 75.60 | 22.22 | 74.86 | 113.39 | 33.33 | 5.82 | 7.20 | 5.73 | 4.76 | 7.20 | 2.12 |
| OPS-A-s-5 | 60 | 10.87 | 13.43 | 47.75 | 55.79 | 22.03 | 71.62 | 83.69 | 33.04 | 6.59 | 6.23 | 5.02 | 5.33 | 6.23 | 2.46 |
| OPS-B-s-1 | 0 | 13.21 | 15.62 | 73.07 | 97.82 | 32.91 | 109.61 | 146.72 | 49.36 | 8.30 | 9.39 | 5.95 | 7.02 | 9.39 | 3.16 |
| OPS-B-s-2 | 13 | 11.34 | 13.99 | 48.88 | 65.45 | 25.01 | 73.32 | 98.18 | 37.51 | 6.47 | 7.02 | 5.80 | 5.24 | 7.02 | 2.68 |
| OPS-B-s-3 | 28 | 12.01 | 14.74 | 63.19 | 63.30 | 27.13 | 94.78 | 94.95 | 40.69 | 7.89 | 6.44 | 5.16 | 6.43 | 6.44 | 2.76 |
| OPS-B-s-4 | 44 | 11.47 | 14.18 | 51.92 | 62.03 | 24.77 | 77.88 | 93.04 | 37.15 | 6.79 | 6.56 | 5.47 | 5.49 | 6.56 | 2.62 |
| OPS-B-s-5 | 60 | 10.7 | 13.28 | 48.24 | 76.73 | 21.09 | 72.36 | 115.09 | 31.64 | 6.76 | 8.67 | 4.68 | 5.45 | 8.67 | 2.38 |
| OPS-C-s-1 | 0 | 14.72 | 15.21 | 69.47 | 104.26 | 37.82 | 134.20 | 156.39 | 56.73 | 9.12 | 10.28 | 6.22 | 8.82 | 10.28 | 3.73 |
| OPS-C-s-2 | 13 | 12.63 | 15.4 | 73.05 | 70.36 | 33.31 | 109.57 | 105.53 | 49.97 | 8.68 | 6.85 | 5.76 | 7.12 | 6.85 | 3.24 |
| OPS-C-s-3 | 28 | 11.43 | 13.86 | 62.47 | 57.66 | 26.36 | 93.71 | 86.49 | 39.54 | 8.20 | 6.24 | 4.82 | 6.76 | 6.24 | 2.85 |
| OPS-C-s-4 | 44 | 10.49 | 12.93 | 52.25 | 56.69 | 25.97 | 78.38 | 85.04 | 38.95 | 7.47 | 6.58 | 5.21 | 6.06 | 6.58 | 3.01 |
| OPS-C-s-5 | 60 | 10.96 | 13.85 | 48.49 | 63.33 | 23.53 | 72.74 | 95.00 | 35.30 | 6.64 | 6.86 | 5.32 | 5.25 | 6.86 | 2.55 |
| | | | | *Active Control* | | | *Active Control* | | | *Active Control* | | | *Active Control* | | |
| OPA-A-s-1 | 0 | | | | | | | | | 8.24 | 9.34 | 5.78 | 7.22 | 9.34 | 3.13 |
| OPA-A-s-2 | 13 | 12.19 | 15.65 | 59.11 | 57.56 | 27.29 | 88.66 | 86.33 | 40.93 | 7.27 | 5.52 | 5.63 | 5.67 | 5.52 | 2.62 |
| OPA-A-s-3 | 28 | 12.5 | 15.33 | 54.93 | 49.84 | 23.15 | 82.39 | 74.76 | 34.72 | 6.59 | 4.88 | 5.27 | 5.37 | 4.88 | 2.26 |
| OPA-A-s-4 | 44 | 12.4 | 15.51 | 46.35 | 48.25 | 18.62 | 69.52 | 72.38 | 27.93 | 5.61 | 4.67 | 4.98 | 4.48 | 4.67 | 1.80 |
| OPA-A-s-5 | 60 | 11.66 | 14.91 | 36.25 | 39.52 | 13.64 | 54.38 | 59.28 | 20.46 | 4.66 | 3.98 | 4.39 | 3.65 | 3.98 | 1.37 |
| OPA-B-s-1 | 0 | | | | | | | | | 8.24 | 9.34 | 5.78 | 7.22 | 9.34 | 3.13 |
| OPA-B-s-2 | 13 | 11.61 | 15 | 56.93 | 55.71 | 24.31 | 85.40 | 83.56 | 36.46 | 7.23 | 5.57 | 5.04 | 5.69 | 5.57 | 2.43 |
| OPA-B-s-3 | 28 | 12.64 | 15.9 | 52.26 | 56.18 | 24.89 | 78.38 | 84.27 | 37.34 | 6.20 | 5.30 | 6.02 | 4.93 | 5.30 | 2.35 |
| OPA-B-s-4 | 44 | 12.88 | 16 | 50.69 | 53.11 | 22.59 | 76.04 | 79.66 | 33.88 | 5.90 | 4.98 | 5.74 | 4.75 | 4.98 | 2.12 |
| OPA-B-s-5 | 60 | 12.43 | 15.47 | 43.75 | 48.13 | 16.55 | 65.62 | 72.19 | 24.83 | 5.28 | 4.67 | 4.70 | 4.24 | 4.67 | 1.60 |
| OPA-C-s-1 | 0 | | | | | | | | | 8.24 | 9.34 | 5.78 | 7.22 | 9.34 | 3.13 |
| OPA-C-s-2 | 13 | 11.6 | 14.69 | 53.27 | 54.50 | 20.29 | 79.91 | 81.74 | 30.43 | 6.89 | 5.56 | 4.42 | 5.44 | 5.56 | 2.07 |
| OPA-C-s-3 | 28 | 11.15 | 13.84 | 45.40 | 44.22 | 21.36 | 68.11 | 66.33 | 32.04 | 6.11 | 4.79 | 5.25 | 4.92 | 4.79 | 2.32 |
| OPA-C-s-4 | 44 | 11.42 | 14.12 | 39.44 | 44.89 | 18.63 | 59.16 | 67.34 | 27.94 | 5.18 | 4.77 | 5.39 | 4.19 | 4.77 | 1.98 |
| OPA-C-s-5 | 60 | 11.32 | 14.82 | 34.81 | 38.51 | 14.48 | 52.21 | 57.77 | 21.72 | 4.61 | 3.90 | 4.71 | 3.52 | 3.90 | 1.47 |
| | | | | *Oxygen* | | | *Oxygen* | | | *Oxygen* | | | *Oxygen* | | |
| OPO-A-s-1 | 0 | | | | | | | | | 8.24 | 9.34 | 5.78 | 7.22 | 9.34 | 3.13 |
| OPO-A-s-2 | 13 | 12.63 | 15.82 | 42.18 | 55.68 | 8.63 | 63.26 | 83.52 | 13.25 | 5.01 | 5.28 | 2.65 | 4.00 | 5.28 | 0.84 |
| OPO-A-s-3 | 28 | 10.93 | 13.51 | 30.84 | 34.84 | 6.23 | 46.41 | 52.25 | 9.35 | 4.25 | 3.87 | 2.20 | 3.44 | 3.87 | 0.69 |
| OPO-A-s-4 | 44 | 12.69 | 14.93 | 26.73 | 33.81 | 4.04 | 40.09 | 50.71 | 6.06 | 3.16 | 3.40 | 1.92 | 2.69 | 3.40 | 0.41 |
| OPO-A-s-5 | 60 | 12.86 | 16.16 | 16.79 | 30.25 | 1.72 | 25.18 | 45.37 | 2.58 | 1.96 | 2.81 | 1.32 | 1.56 | 2.81 | 0.16 |
| OPO-B-s-1 | 0 | | | | | | | | | 8.24 | 9.34 | 5.78 | 7.22 | 9.34 | 3.13 |
| OPO-B-s-2 | 13 | 15.01 | 18.52 | 52.32 | 68.67 | 8.70 | 78.48 | 103.00 | 13.05 | 5.23 | 5.56 | 2.50 | 4.24 | 5.56 | 0.70 |
| OPO-B-s-3 | 28 | 11.61 | 14.61 | 38.16 | 40.25 | 7.35 | 57.24 | 60.37 | 11.02 | 4.85 | 4.13 | 2.27 | 3.92 | 4.13 | 0.75 |
| OPO-B-s-4 | 44 | 12.17 | 14.92 | 26.02 | 28.88 | 3.32 | 39.03 | 43.33 | 4.98 | 3.21 | 2.90 | 1.55 | 2.62 | 2.90 | 0.33 |
| OPO-B-s-5 | 60 | 10.74 | 13.41 | 14.36 | 20.59 | 1.67 | 21.54 | 30.89 | 2.51 | 2.01 | 2.30 | 1.25 | 1.61 | 2.30 | 0.19 |
| OPO-C-s-1 | 0 | | | | | | | | | 8.24 | 9.34 | 5.78 | 7.22 | 9.34 | 3.13 |
| OPO-C-s-2 | 13 | 11.47 | 14.46 | 35.41 | 57.55 | 7.08 | 53.11 | 86.33 | 10.62 | 4.63 | 5.97 | 2.29 | 3.67 | 5.97 | 0.73 |
| OPO-C-s-3 | 28 | 11.94 | 14.74 | 36.79 | 39.45 | 7.11 | 55.18 | 59.18 | 10.66 | 4.62 | 4.02 | 2.31 | 3.74 | 4.02 | 0.72 |
| OPO-C-s-4 | 44 | 11.37 | 15.38 | 21.49 | 29.96 | 2.89 | 32.23 | 44.94 | 4.33 | 2.83 | 2.92 | 1.53 | 2.10 | 2.92 | 0.28 |
| OPO-C-s-5 | 60 | 11.92 | 14.87 | 10.60 | 20.04 | 1.63 | 15.89 | 30.05 | 2.44 | 1.33 | 2.02 | 1.83 | 1.07 | 2.02 | 0.16 |
| | | | | *Oxygen/Nutrients* | | | *Oxygen/Nutrients* | | | *Oxygen/Nutrients* | | | *Oxygen/Nutrients* | | |
| OPON-A-s-1 | 0 | | | | | | | | | 8.24 | 9.34 | 5.78 | 7.22 | 9.34 | 3.13 |
| OPON-A-s-2 | 13 | 12.01 | 14.92 | 35.73 | 37.37 | 5.82 | 53.59 | 56.05 | 8.72 | 4.46 | 3.76 | 1.95 | 3.59 | 3.76 | 0.58 |
| OPON-A-s-3 | 28 | 11.61 | 14.34 | 23.07 | 37.98 | 3.45 | 34.60 | 56.97 | 5.18 | 3.01 | 3.97 | 1.72 | 2.41 | 3.97 | 0.36 |
| OPON-A-s-4 | 44 | 12.17 | 15.74 | 13.03 | 28.55 | 1.46 | 19.55 | 42.82 | 2.20 | 1.61 | 2.72 | 1.37 | 1.24 | 2.72 | 0.14 |
| OPON-A-s-5 | 60 | 9.79 | 12.42 | 6.15 | 15.27 | 0.69 | 9.23 | 22.90 | 1.03 | 0.94 | 1.84 | 1.09 | 0.74 | 1.84 | 0.08 |
| OPON-B-s-1 | 0 | | | | | | | | | 8.24 | 9.34 | 5.78 | 7.22 | 9.34 | 3.13 |
| OPON-B-s-2 | 13 | 13.13 | 15.88 | 35.58 | 37.80 | 9.37 | 54.85 | 58.70 | 14.06 | 4.18 | 3.57 | 3.37 | 3.45 | 3.57 | 0.89 |
| OPON-B-s-3 | 28 | 11.65 | 14.4 | 24.63 | 25.40 | 5.14 | 36.94 | 38.10 | 7.71 | 3.17 | 2.65 | 2.43 | 2.57 | 2.65 | 0.54 |
| OPON-B-s-4 | 44 | 12.47 | 15.41 | 11.68 | 24.83 | 1.82 | 17.53 | 37.25 | 2.73 | 1.41 | 2.42 | 1.94 | 1.14 | 2.42 | 0.18 |
| OPON-B-s-5 | 60 | 11.85 | 14.79 | 8.00 | 13.47 | 0.50 | 9.00 | 20.21 | 0.76 | 0.76 | 1.37 | 1.00 | 0.61 | 1.37 | 0.05 |
| OPON-C-s-1 | 0 | | | | | | | | | 8.24 | 9.34 | 5.78 | 7.22 | 9.34 | 3.13 |
| OPON-C-s-2 | 13 | 11.8 | 14.69 | 42.38 | 37.74 | 9.69 | 63.57 | 56.61 | 14.84 | 5.39 | 3.80 | 2.75 | 4.27 | 3.80 | 1.00 |
| OPON-C-s-3 | 28 | 12.65 | 15.62 | 27.55 | 27.58 | 3.85 | 41.33 | 41.37 | 5.77 | 3.27 | 2.65 | 1.77 | 2.65 | 2.65 | 0.37 |
| OPON-C-s-4 | 44 | 12.69 | 14.34 | 16.49 | 19.81 | 1.69 | 24.73 | 29.71 | 2.53 | 1.95 | 2.07 | 1.30 | 1.72 | 2.07 | 0.18 |
| OPON-C-s-5 | 60 | 12.4 | 15.71 | 7.37 | 14.81 | 0.61 | 11.06 | 22.22 | 0.92 | 0.89 | 1.41 | 1.03 | 0.70 | 1.41 | 0.06 |

Note[1] : Analysis by GC/FID. Confirmation of analytes and retention times confirmed by GCMS under identical chromatographic conditions.

## Table A-2. Summary of Gas Chromatographic/FID Analytical Raw Data for Respirometer Test # 3

| SAMPLE | DAY | SAMPLE DRY WEIGHT (mg) | SAMPLE WET WEIGHT (mg) | [1]EXTRACT CONCENTRATION PAH (ug/ml) | | | TOTAL EXTRACTED MASS PAH (ug) | | | *PAH CONCENTRATION D.W. (ug/mg)* | | | *PAH CONCENTRATION W.W. (ug/mg)* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | NP | 2-MNP | PHN | NP | 2-MNP | PHN | NP | 2-MNP | PHN | NP | 2-MNP | PHN |
| | | | | ***Sterile Control*** | | | ***Sterile Control*** | | | ***Sterile Control*** | | | ***Sterile Control*** | | |
| OPS-0-S | 0 | 4.75 | 6.25 | 125.96 | 206.26 | 285.12 | 188.94 | 309.38 | 477.68 | 33.34 | 51.64 | 83.47 | 25.87 | 40.64 | 64.92 |
| OPS-1-S | 15 | 3.56 | 5.06 | 75.08 | 81.43 | 179.58 | 112.62 | 122.15 | 269.37 | 31.64 | 34.31 | 75.67 | 22.26 | 24.14 | 53.23 |
| OPS-2-S | 29 | 6.32 | 7.91 | 91.04 | 115.15 | 205.44 | 136.55 | 172.73 | 308.16 | 21.61 | 27.33 | 48.76 | 17.26 | 21.84 | 38.96 |
| OPS-3-S | 41 | 6.79 | 8.34 | 211.76 | 422.19 | 570.34 | 317.64 | 633.28 | 855.50 | 46.78 | 93.27 | 125.99 | 38.09 | 75.93 | 102.58 |
| | | | | ***Active*** | | | ***Active*** | | | ***Active*** | | | ***Active*** | | |
| | 0 | | | | | | | | | 33.34 | 51.64 | 83.47 | 25.96 | 40.73 | 55.14 |
| OPA-1-S | 15 | 4.32 | 5.38 | 20.92 | 22.80 | 170.93 | 31.38 | 34.20 | 256.40 | 7.26 | 7.92 | 59.35 | 5.83 | 6.36 | 47.66 |
| *OPA-2-S* | 29 | *8.64* | *10.76* | *28.60* | *131.08* | *233.08* | 42.90 | *196.62* | *349.62* | 4.97 | *22.76* | *40.47* | 3.99 | 18.27 | 32.49 |
| OPA-3-S | 41 | 6.30 | 7.85 | 25.54 | 112.96 | 152.74 | 38.30 | 169.44 | 229.11 | 6.08 | 26.90 | 36.37 | 4.88 | 21.58 | 29.19 |
| | | | | ***Oxygen*** | | | ***Oxygen*** | | | ***Oxygen*** | | | ***Oxygen*** | | |
| | 0 | | | | | | | | | 33.34 | 51.64 | 83.47 | 25.96 | 40.73 | 55.14 |
| OPO-1-S | 15 | 5.60 | 8.28 | 25.21 | 101.30 | 135.97 | 37.82 | 151.95 | 203.96 | 6.75 | 27.13 | 36.42 | 4.57 | 18.35 | 24.63 |
| *OPO-2-S* | 29 | *8.61* | *10.76* | *0.70* | *45.76* | *191.22* | ***0.70*** | *68.64* | *286.83* | ***0.70*** | 7.97 | *33.32* | ***0.70*** | 6.38 | 26.66 |
| OPO-3-S | 41 | 6.66 | 7.85 | 0.70 | 0.70 | 82.20 | **0.70** | **0.60** | 123.30 | **0.70** | **0.70** | 18.51 | **0.70** | **0.70** | 15.71 |

NOTE[(1)]: Analysis by GC/FID. Confirmation of analytes and retention times performed by GCMS under identical chromatographic conditions.

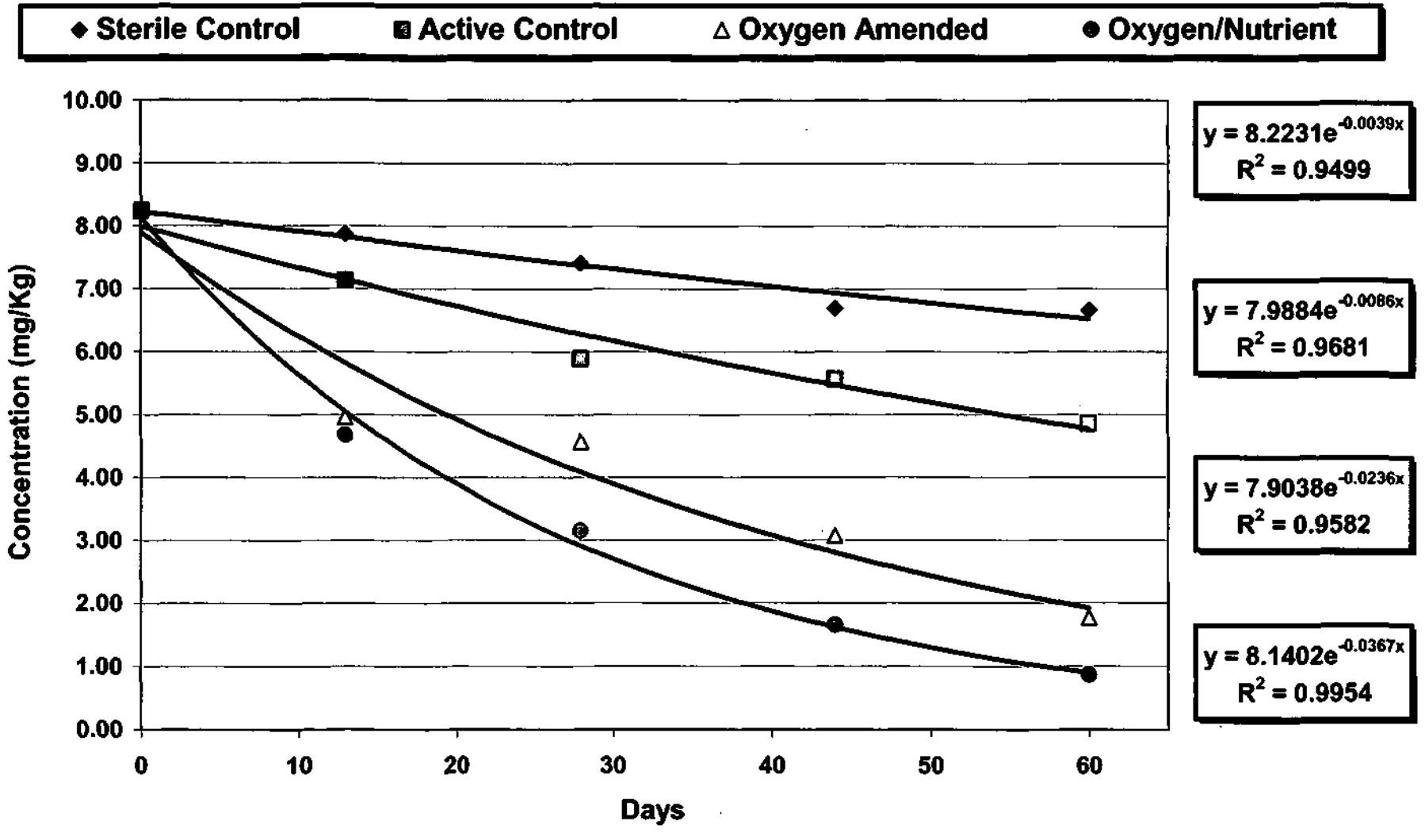
**Table A-3. Summary of GC/PID Analytical Raw Data for Aerobic Vinyl Chloride Microcosm Test**

| Sample | Date | Time (Days) | Vinyl Chloride Concentration (ug/L) | | Data Statistics | | |
|---|---|---|---|---|---|---|---|
| | | | Headspace | Liquid | | | |
| SCA | 12/10/2002 | 0 | 45093.78 | 982.44 | | | |
| | 12/12/2002 | 2 | 48000.31 | 969.70 | | | |
| | 12/16/2002 | 6 | 49500.19 | 1000.00 | | | |
| | 12/28/2002 | 16 | 5713.74 | 115.43 | | | |
| | 1/5/2003 | 24 | 30192.77 | 609.96 | | | |
| | | | | | **Sterile Control Microcosms** | | |
| SCB | 12/10/2002 | 0 | 48322.00 | 1052.77 | *Average* | *STDEV* | *95% CI* |
| | 12/12/2002 | 2 | 18127.43 | 366.21 | 1118.20 | 177.76 | 201.15 |
| | 12/16/2002 | 6 | 10613.06 | 214.41 | 910.99 | 517.92 | 586.07 |
| | 12/28/2002 | 16 | | | 600.16 | 392.99 | 444.70 |
| | 1/5/2003 | 24 | 31954.87 | 645.55 | 83.58 | 45.05 | 50.97 |
| | | | | | 627.90 | 17.80 | 20.14 |
| SCC | 12/10/2002 | 0 | 65310.28 | 1319.40 | | | |
| | 12/12/2002 | 2 | 69153.91 | 1397.05 | | | |
| | 12/16/2002 | 6 | 29010.13 | 586.06 | | | |
| | 12/28/2002 | 16 | 2560.40 | 51.73 | | | |
| | 1/5/2003 | 24 | 31096.00 | 628.20 | | | |
| ACTA | 12/10/2002 | 0 | 50884.82 | 1027.98 | | | |
| | 12/12/2002 | 2 | 31556.32 | 637.50 | | | |
| | 12/16/2002 | 6 | 336.82 | 6.80 | | | |
| | 12/28/2002 | 16 | 201.63 | 4.07 | | | |
| | 1/5/2003 | 24 | 324.65 | 6.56 | | | |
| | | | | | **Unamended Microcosms** | | |
| ACTB | 12/10/2002 | 0 | 49112.78 | 992.18 | *Average* | *STDEV* | *95% CI* |
| | 12/12/2002 | 2 | 51260.39 | 1035.56 | 1013.50 | 18.86 | 21.34 |
| | 12/16/2002 | 6 | 2555.92 | 51.63 | 744.22 | 255.30 | 288.89 |
| | 12/28/2002 | 16 | 1015.16 | 20.51 | 29.22 | 31.70 | 35.87 |
| | 1/5/2003 | 24 | 509.36 | 10.29 | 10.52 | 8.77 | 9.93 |
| | | | | | 7.32 | 2.67 | 3.02 |
| ACTC | 12/10/2002 | 0 | 50507.77 | 1020.36 | | | |
| | 12/12/2002 | 2 | 27700.00 | 559.60 | | | |
| | 12/16/2002 | 6 | | | | | |
| | 12/28/2002 | 16 | 345.18 | 6.97 | | | |
| | 1/5/2003 | 24 | 252.95 | 5.11 | | | |
| OXYA | 12/10/2002 | 0 | 50026.72 | 1010.64 | | | |
| | 12/12/2002 | 2 | 48258.00 | 974.91 | | | |
| | 12/16/2002 | 6 | 1504.15 | 30.39 | | | |
| | 12/28/2002 | 16 | 3.83 | 0.08 | | | |
| | 1/5/2003 | 24 | 2.66 | 0.05 | | | |
| | | | | | **Oxygen Amended Microcosms** | | |
| OXYB | 12/10/2002 | 0 | 49707.41 | 1004.19 | *Average* | *STDEV* | *95% CI* |
| | 12/12/2002 | 2 | 33517.82 | 677.13 | 1014.12 | 12.05 | 13.63 |
| | 12/16/2002 | 6 | 2728.27 | 55.12 | 737.21 | 214.08 | 242.25 |
| | 12/28/2002 | 16 | 3.90 | 0.08 | 45.38 | 13.17 | 14.91 |
| | 1/5/2003 | 24 | 2.54 | 0.05 | 0.08 | 0.0021 | 0.0023 |
| | | | | | 0.05 | 0.0018 | 0.0020 |
| OXYC | 12/10/2002 | 0 | 50862.26 | 1027.52 | | | |
| | 12/12/2002 | 2 | 27700.00 | 559.60 | | | |
| | 12/16/2002 | 6 | 2505.87 | 50.62 | | | |
| | 12/28/2002 | 16 | 4.03 | 0.08 | | | |
| | 1/5/2003 | 24 | 2.71 | 0.05 | | | |

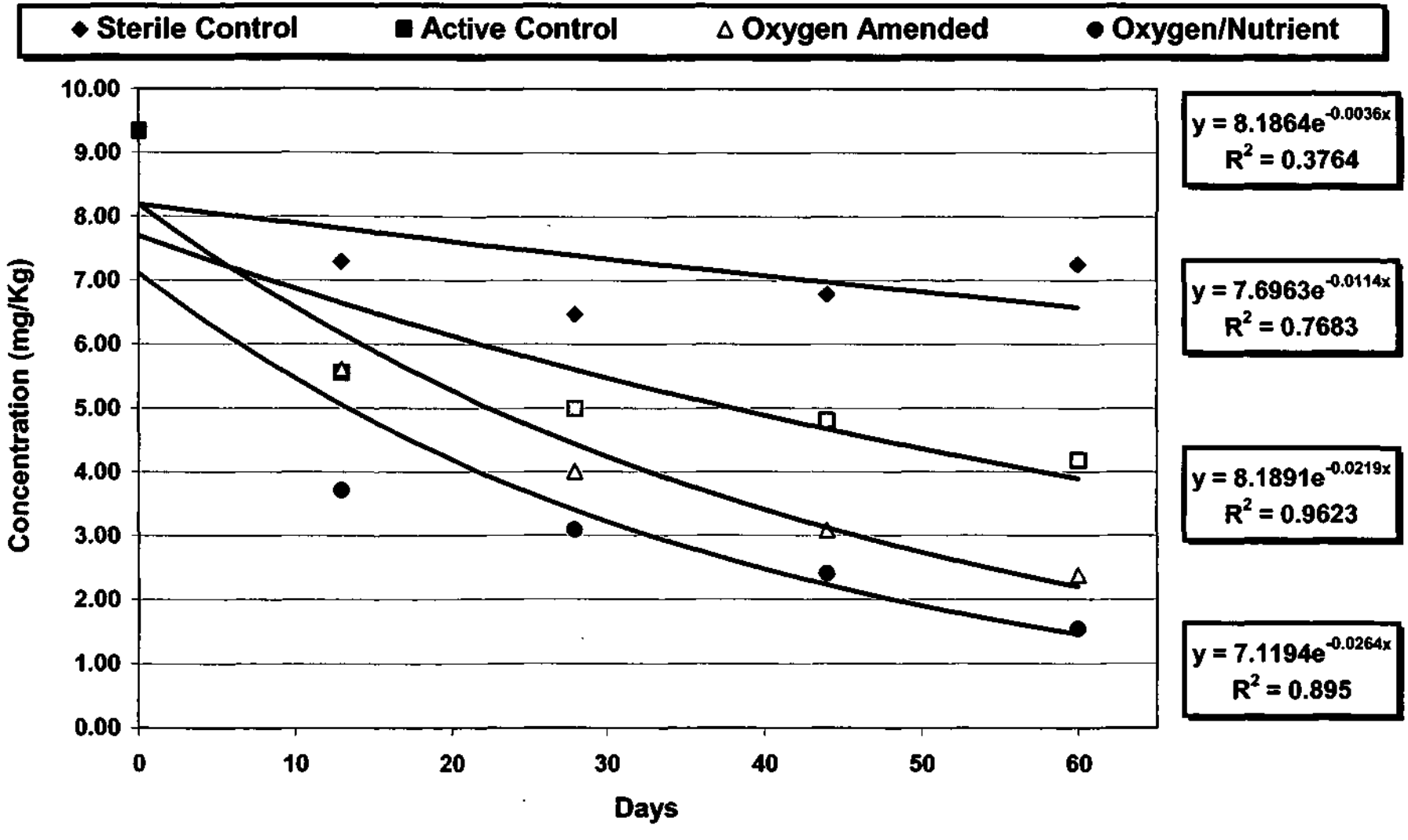
# APPENDIX B

## Respirometer: PAH Test #2 – Supplemental Figure's 4-a to 4-c

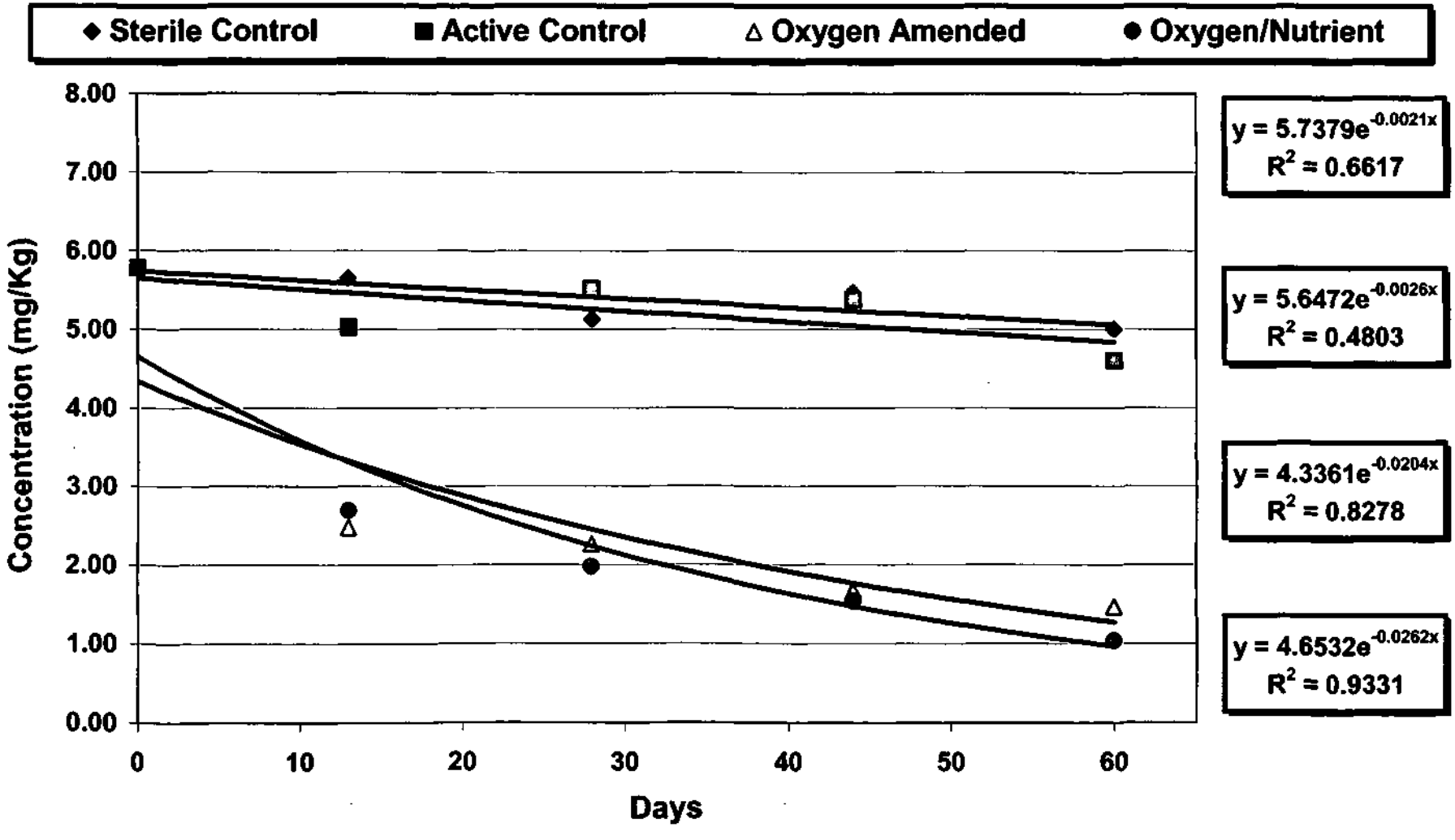
## Figure 4-a. Naphthalene Biodegradation Respirometer: PAH Test #2



## Figure 4-b. 2-Methylnaphthalene Biodegradation During Respirometer: PAH Test #2



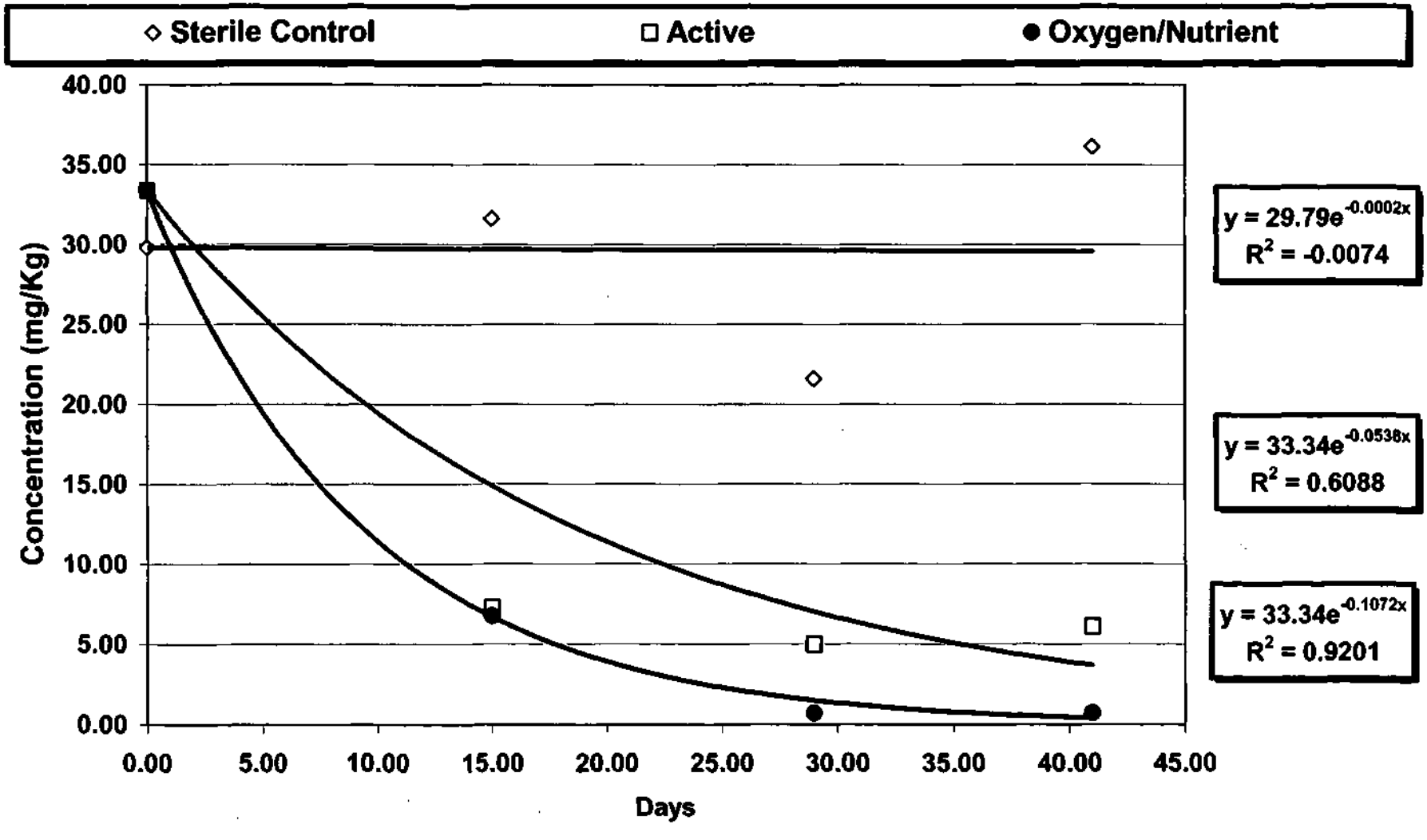
## Figure 4-c. Phenanthrene Biodegradation Trends During Respirometer: PAH Test #2



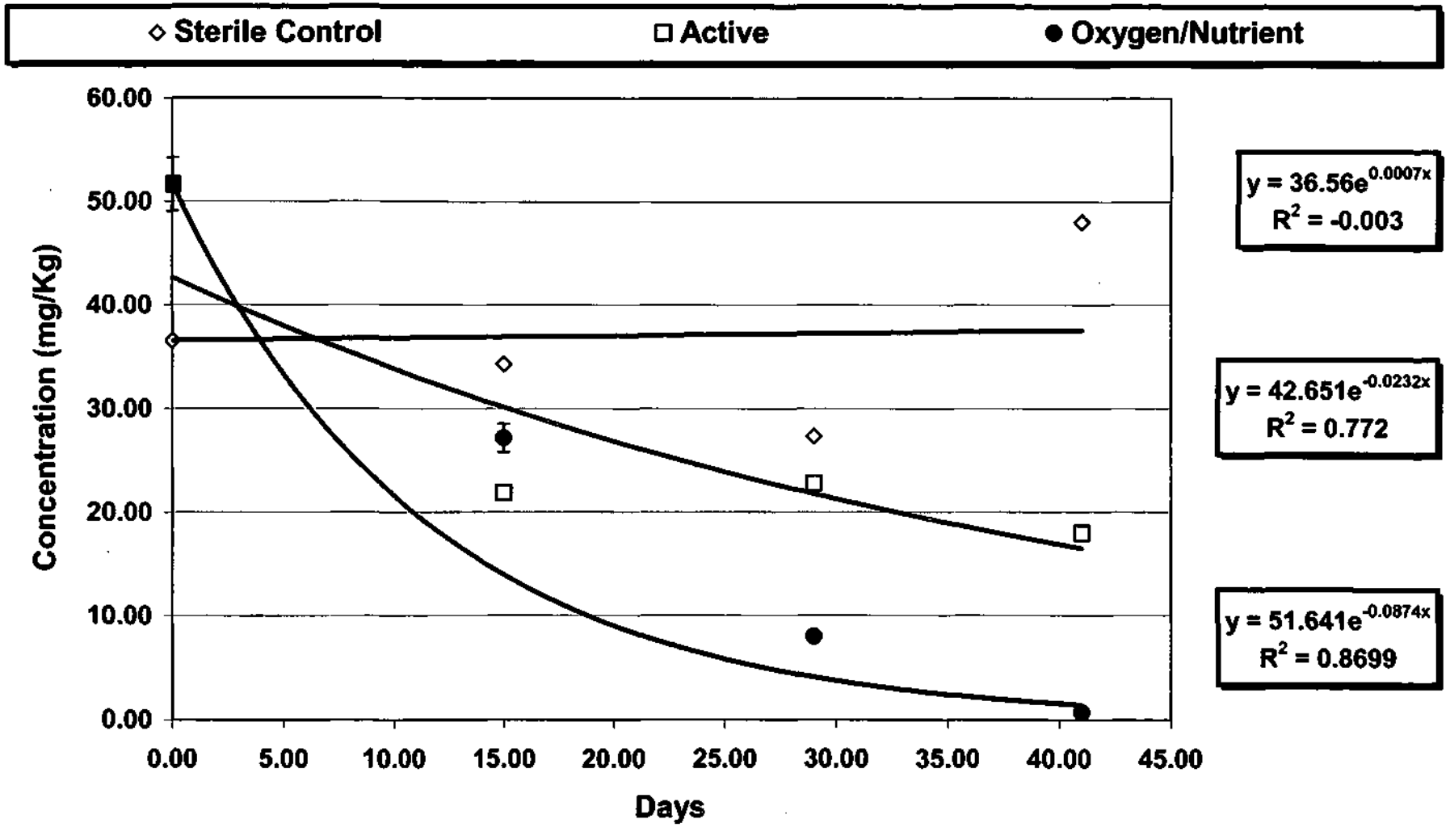
# APPENDIX C

## Respirometer: PAH Test #3 – Supplemental Figure's 6-a to 6-c

## Figure 6-a. Naphthalene Biodegradation Trends During Respirometer: PAH Test #3



## Figure 6-b. 2-Methylnaphthalene Biodegradation Trends During Respirometer: PAH Test #3



# Figure 6-c. Phenanthrene Biodegradation Trends During Respirometer: PAH Test #3